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Advances in *Chlorella* microalgae for sustainable wastewater treatment and bioproduction

Yazan Abuhasheesh [a,b,1], Aya Ghazal [a,b,1], Doris Ying Ying Tang [a], Fawzi Banat [a,b,c], Shadi W. Hasan [a,b], Pau Loke Show [a,c,*]

[a] Department of Chemical and Petroleum Engineering, Khalifa University, P.O. Box 127788, Abu Dhabi, United Arab Emirates
[b] Center for Membranes and Advanced Water Technology (CMAT), Khalifa University of Science and Technology, PO Box 127788, Abu Dhabi, United Arab Emirates
[c] Food Security and Technology Center, Khalifa University, P.O. Box 127788, Abu Dhabi, United Arab Emirates

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ABSTRACT

The rising demand for effective and sustainable wastewater treatment strategies initiated a growing interest in treating wastewater with microalgal-based processes, especially *Chlorella* microalgae. Phycoremediation has been confirmed to be a highly effective method to efficiently remove numerous pollutants from wastewater, including industrial, municipal, household, and agricultural wastewater. This review discusses the dual role of *Chlorella* in both wastewater treatment and bioproduction, focusing on recent studies between 2019 and 2024. It emphasizes the ability of its different species to efficiently remediate various pollutants and the conversion of their biomass into a variety of valuable products, contributing to the circular bioeconomy. Furthermore, this review highlights the crucial role of incorporating artificial intelligence (AI) in optimizing various conditions and parameters to enhance the phycoremediation and production processes to achieve greater cost-effectiveness. Ongoing research efforts aim to improve microalgae properties through synthetic biology, link them with other microorganisms, and incorporate cutting-edge technologies. These initiatives seek to accelerate the commercialization of microalgae for wastewater bioremediation and bioproduction, thereby contributing to a more sustainable future.

1. Introduction

Global water scarcity has emerged as a significant concern. Improper water management, growth in population, climate change, and other natural factors are the main factors that contribute to water scarcity [1]. By 2030, the world is expected to confront a substantial 40 % deficit in water, as the United Nations secretary proclaimed in 2017 [2]. The treatment of contaminated wastewater from pollutants is crucial to overcome the scarcity of clean water sources and safeguard the environment. The expansion of industrial practices and human activities generates vast amounts of wastewater containing diverse organic, inorganic, and suspended and dissolved solid pollutants [3]. These pollutants include heavy metals, oil, and emerging contaminants such as pharmaceuticals and synthetic dyes. Poor management and handling of discharged wastewater can cause adverse effects on the environment, water sources, aquatic life, and human health. Hence, proper treatment of generated wastewater is critical.

Wastewater treatment plants have historically relied on various conventional methods, including coagulation-flocculation, sedimentation, chemical precipitation, disinfection, aerobic activated sludge, and anaerobic digestion [4]. However, these approaches have been criticized for their high energy requirements and expensive capital and operational expenses [5]. Additionally, the environmental impact of conventional technologies is highlighted by the fact that they account for 4 % of greenhouse gas emissions [6]. Emerging technologies, such as membrane technology, have shown great potential in effectively eliminating various contaminants from wastewater. However, membrane technology encounters a major drawback in the formation of membrane fouling, causing its poor reusability [7].

Microalgae-based processes stand out as an advanced and sustainable technology for wastewater remediation. The utilization of microalgae proved its potential as an affordable and eco-friendly tool with high capacity and removal efficiency of emerging contaminants, such as persistent organics, pharmaceuticals, microplastics, and endocrine

* Corresponding author.
E-mail address: PauLoke.Show@ku.ac.ae (P.L. Show).
[1] Authors share first authorship

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disruptors [8]. Microalgae are unique in their ability to thrive in wastewater environments by consuming the present organic and inorganic contaminants as a source of nutrients [9]. It can also be essential as a biological carbon dioxide (CO_2) fixation method as it utilizes the CO_2 from wastewater and generates O_2 through photosynthesis [8]. This dual capability of pollutant removal and O_2 generation is crucial as it produces clean water and reduces the carbon footprint of wastewater treatment processes. Furthermore, algal biomass is formed during the bioremediation of wastewater via microalgae. Algal biomass can be employed to prepare diverse bioproducts, including biofuels, biofertilizers, bioplastics, and livestock feed. The various pros of integrating microalgae render it a promising and sustainable solution to enhance water quality and green resource production.

The main types of microalgae include *Chlorella, Scenedesmus, Spirulina, Chlamydomonas, Desmodesmus*, and *Nannochloropsis* [10]. *Chlorella* is widely employed due to its elevated removal efficiency of contaminants, fast growth rate and biomass productivity, and resistance to toxic substances and environments [11]. In addition, it possesses a high capability to accumulate lipids, which makes it a suitable source for biofuel production [12]. Employing *Chlorella* for wastewater treatment was not limited to being a primary remediation approach but also an additive to enhance the performance of other technologies. For instance, *Chlorella* can be incorporated into nanomaterials and hydrogel beads to produce adsorbents [13,14]. Moreover, it can be used in algal membrane bioreactors and bioelectrochemical systems [15]. Another approach for utilizing microalgae is the preparation of a microalgal consortium, by which *Chlorella* can coexist with other species of microalgae, bacteria, or fungi in the same environment. Microalgal consortiums are considered more stable, more effective, and possess higher biomass yield and lipid content [16]. Several challenges can be associated with utilizing microalgae for wastewater treatment. The way diverse kinds of microalgae react and their performance in removing pollutants varies in accordance with the wastewater source [9]. In addition to the characteristics of wastewater, the treatment process can be influenced by other factors. These include the cultivation mode, light intensity, and nutrient and CO_2 availability [17]. Harsh environments with high concentrations of total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), biological oxygen demand (BOD), and some heavy metals (i.e., Cd, Hg) can be toxic to microalgae and affect its stability [18,19].

Integrating artificial intelligence (AI) and machine learning (ML) algorithms can effectively enhance the efficiency of microalgae-based systems for wastewater treatment and resource recovery. They can be employed to monitor and optimize various parameters and conditions affecting the system and identify microalgae cultures [20]. Several parameters influencing microalgae growth and its performance, including nutrient availability, medium type and characteristics, temperature, pH, and light intensity, can be optimized using AI and ML [21]. The harvested microalgal biomass can be converted into biofuels, biohydrogen, and biofertilizers via different conversion processes [22]. AI and ML algorithms are vital in improving such processes while saving huge expenses and time. The temperature, reaction time, catalyst loading, and other process variables can be optimized to maximize bioproduction yields [20].

This review spotlights the application of *Chlorella* microalgae in wastewater treatment and bioproduction. The primary aim is to provide a critical overview of the recent advances and current state-of-the-art utilizing several species of *Chlorella* to eliminate contaminants from wastewater, including heavy metals, nutrients, pharmaceuticals, dyes, pathogens, and oil. To the best of the authors' knowledge, no reviews focus on the performance of *Chlorella* specifically for wastewater bioremediation, bioproduction, and AI-assisted technologies. The main mechanisms for sustainable wastewater bioremediation via microalgae are discussed alongside common challenges that face such processes. Furthermore, the utilization of the harvested biomass after the treatment process to generate biofuel and other bioproducts is covered. This review highlights the implementation of AI and ML algorithms to assess and predict the performance of microalgae-based wastewater treatment processes and the bioproduction of distinct biofuels and biofertilizers using the produced biomass.

2. *Chlorella* microalgae contribution to sustainable wastewater treatment

According to *Scopus*, the number of studies on microalgae utilization with wastewater and the most employed types of microalgae for wastewater treatment and bioproduction in the past decade are presented in Fig. 1(a) and (b), respectively. Among species used for wastewater bioremediation, *Chlorella* was the most utilized, as illustrated in Fig. 1(b). Furthermore, various strains from *Chlorella* microalgae, such as *vulgaris, sorokiniana, pyrenoidosa*, and *minutissima*, have been studied recently to test their potential (Fig. 1(c)). The isolated strains that cannot be determined to what specific strain of *Chlorella* they belong are named *Chlorella* sp. Hence, the strains of *Chlorella* sp. mentioned in the studies may differ. Tremendous amounts of nutrients, including N and P, available in wastewater produced from industrial, domestic, and agricultural activities can seriously threaten the ecosystem balance, causing eutrophication [23]. N, P, and carbon are essential nutrients for microalgae growth and function. As a result, the microalgae assimilation process is strongly affected by the composition and nutrient profile of the cultivation medium [24].

2.1. Microalgae cultivation modes

Chlorella hold the potential to be cultivated in distinct types of wastewater and biomass, allowing them to be a great source of biofuels, healthy food, and fertilizers [25]. Cultivating microalgae necessitates substantial quantities of water, inorganic nutrients, and CO_2. As a result, microalgae cultivation with fresh water, N and P fertilizers, and purchased CO_2 makes the process highly expensive. Instead, using distinct wastewater types and flue gas is a convenient alternative for sustainably cultivating microalgae and overcoming the high costs of conventional cultivation. This maintains an economically feasible culture medium for microalgal growth while simultaneously reducing the levels of organic and inorganic contaminants in the wastewater, hence formulating a biological wastewater treatment method [26,27]. There are three microalgae cultivation modes, namely autotrophic, heterotrophic, and mixotrophic (Fig. 2). The distinction between those methods is based on the source of carbon and energy. The advantages and limitations of these cultivation modes are discussed in Table 1.

Autotroph utilizes the available sunlight as an energy source and CO_2 as a carbon source, making it vital in sequestrating CO_2 from the atmosphere [28]. Autotrophic algae transform solar energy into biomass using photosynthetic reactions [29]. On the other hand, heterotrophy is a light-independent microalgae cultivation mode that uses organic sources solely as energy and carbon sources. Despite the unnecessity of illumination, only certain microalgae strains can suit heterotrophic cultivation mode, making the selection criteria of microalgal strains for cultivation a vital factor [30]. Under mixotrophic conditions, microalgae estimate energy sources from light and organic carbon while utilizing CO_2 as the carbon source. Mixotrophic cultivation merges the merits of autotrophy and heterotrophy. The mixotrophic growing conditions allow the microalgae to utilize organic carbon to grow heterotrophically, which enhances the growth rate and boosts lipid and biomass accumulation, along with performing photosynthesis reactions that sequestrate CO_2 and produce O_2. Consequently, this significantly reduces overall CO_2 emission compared to the heterotrophic mode [31].

2.2. Phycoremediation mechanisms for wastewater treatment

Phycoremediation is the process of using macroalgae or microalgae to remove or biotransform various contaminants and their toxicity [30]. Microalgae are employed to reduce levels of distinct pollution through

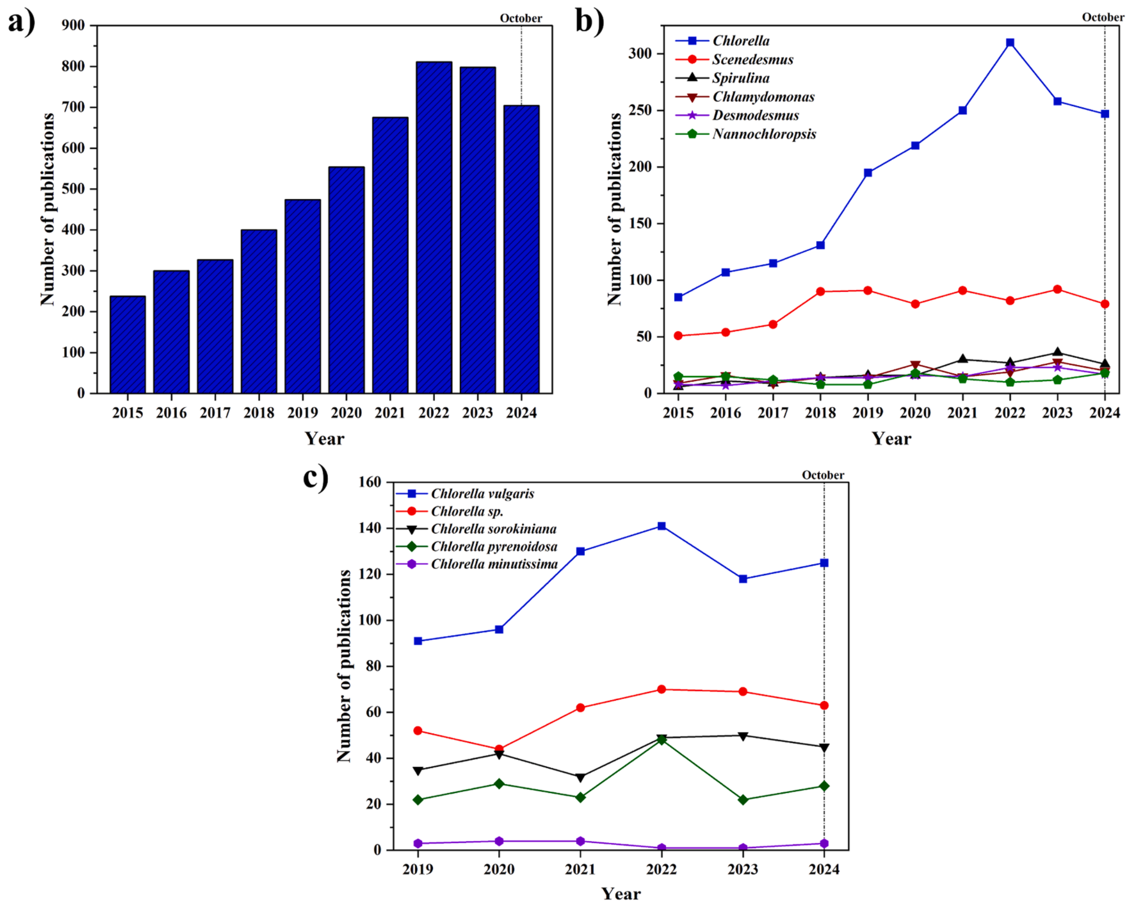

Fig. 1. The number of research publications, based on Scopus, a) between 2015 and 2024 for using microalgae with wastewater. Keywords: Microalgae AND wastewater. b) different types of microalgae for wastewater treatment. Keywords: Microalgae AND Chlorella/ Scenedesmus/… AND wastewater. c) between 2019 and 2024 for utilizing different Chlorella microalgae species for wastewater treatment. Keywords: Microalgae AND Chlorella AND vulgaris/ sp./… AND wastewater.

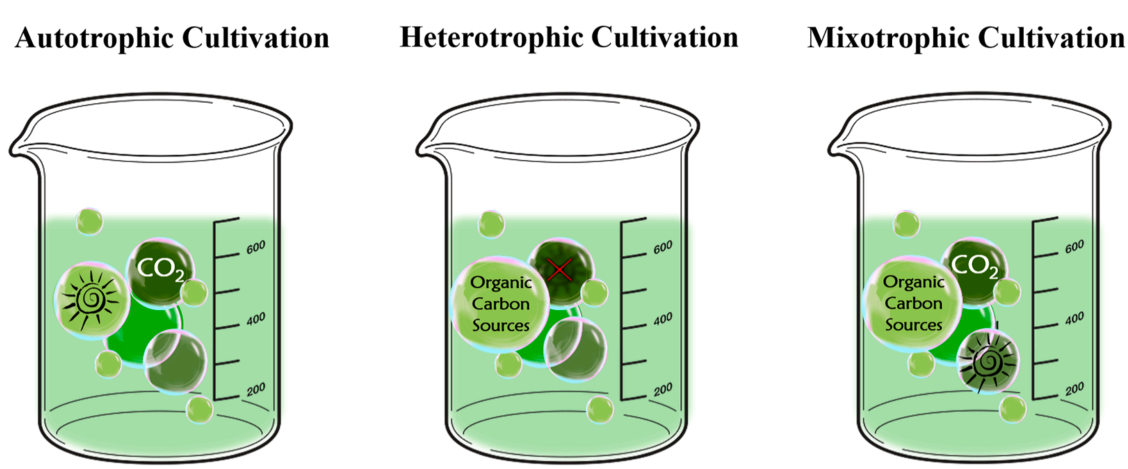

Fig. 2. Main cultivation modes of microalgae and their requirements.

different mechanisms, including biosorption, bioaccumulation, and biodegradation, as demonstrated in Fig. 3.

Biosorption is a metabolic independent mechanism that encounters an interaction between contaminants (positively charged) and microalgae's cell walls or secretions (negatively charged). This illustrates the efficacy of such mechanisms in eliminating emerging cationic lipophilic contaminants and heavy metals [32]. Biosorption is acknowledged as an inactive process, considering the sorbent to be a biological material capable of attaching and concentrating contaminants from water bodies. The biological material involved in such a mechanism could be dead or living microorganisms and their constituents. Precipitation, ion exchange, adsorption, absorption, surface complexation, and electrostatic interaction are all mechanisms that support chemical, physical, and non-metabolic processes related to the biosorption technique [33,34].

The efficacy of the biosorption process is significantly impacted by pollutants' functional and structural compositions in addition to the affinity of the water. Due to electrostatic interactions, lower affinity species feature greater adsorption within microalgae cells serving as

Table 1
Comparison among distinct types of microalgal cultivation modes.

Cultivation mode	Energy source	Carbon source	Advantages	Limitations
Autotrophic	Sunlight	CO_2	– Captures CO_2 from emission sources – Transforms solar energy into biomass using photosynthesis – Economically feasible in open ponds or closed photobioreactors	– Cost issues – Light exposure dependence
Heterotrophic	Organic sources	Organic sources	– Light-independent – Uses solely organic sources for energy and carbon – Allows microalgae to switch metabolism based on carbon or nutrient availability, adapting to harsh conditions	– Limited to specific microalgae strains – Requires suitable organic or nutrient sources
Mixotrophic	Light and organic carbon	CO_2 and organic carbon	– Combines benefits of autotrophy and heterotrophy – Enhance growth rate, lipid, and biomass accumulation – Reduces CO_2 emissions; provides flexibility in harsh conditions	– Complexity in maintaining optimal conditions

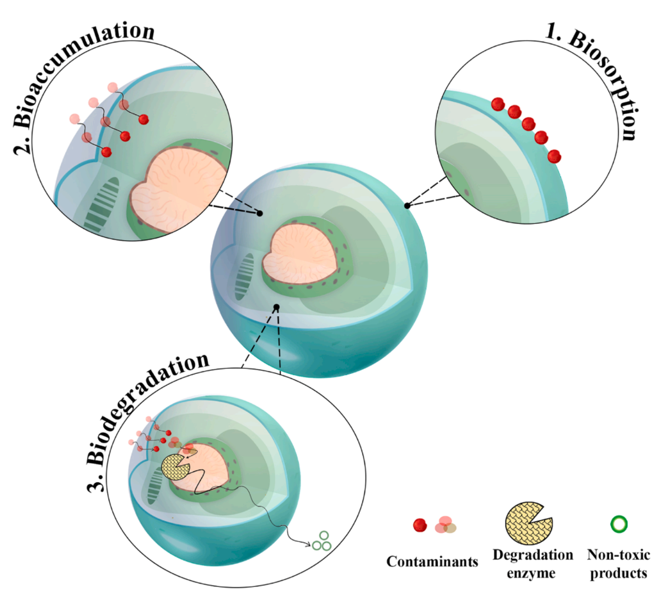

Fig. 3. Microalgae phycoremediation mechanisms of pollutants.

hyper-adsorbents. Meanwhile, hydrophilic organic pollutants are persistent in aquatic bodies and possess low adsorption [35]. Gojkovic er al. [36] emphasized an efficient removal of distinct hydrophobic pharmaceutical drugs like biperiden, trihexyphenidyl, clomipramine, and amitriptyline with biosorption rates above 70 %. Nevertheless, an average amount of 60 % of hydrophilic compounds, including caffeine, fluconazole, trimethoprim, codeine, carbamazepine, oxazepam, and tramadol, persist in water, showing low removal rates.

In contrast with the passive biosorption process, bioaccumulation is an active process stimulated by energy requirements [37]. Organic and inorganic pollutants, such as nitrates, phosphates, sulfates, pesticides, and heavy metals, are transported inside the living cells and eradicated from water bodies, limiting bioaccumulation only to living cells [34,38]. During microalgae growth, along with nutrients, pollutants are actively absorbed and accumulated inside the algal cell. Bioaccumulation processes are identified to be slower and more complex than biosorption ones [19]. There are three main mechanisms for bioaccumulation across microalgae cell walls, namely, passive diffusion, passive facilitated diffusion, and active (energy-dependent) uptake [39]. Bioaccumulation using *Chlorella sorokiniana* was the principal removal mechanism for removing azo dye from wastewater with higher than 70 % decolorization in the study of Tarbajova et al. [40]. Results of Xiong et al. [41] showcased the enhanced removal capability of levofloxacin using *Chlorella vulgaris*, highlighting bioaccumulation as the primary removal mechanism followed by biodegradation.

Biodegradation uses enzymes produced by microorganisms to catalyze a process of metabolic degradation or break down of toxic, complex chemical compounds into less toxic simpler compounds. Biodegradation is an essential primary process that accounts for the elimination of contaminants from wastewater, including the majority of organics, and utilizes them as nutrients for microalgae growth [42,43]. The criteria of biodegradation differ from the simple biological filtration process implemented in biosorption or bioaccumulation as it either breakdown contaminants by complete mineralization of complex compounds into CO_2 and H_2O or by encountering enzymatic reactions series to generate distinct metabolic intermediates, in other words, biotransformation. This justifies it as one of the most promising microalgae processes for the remediation of pollutants. Additionally, challenges related to the disposal of the microalgal biomass produced during biosorption or bioaccumulation processes are defeated using mechanisms of biodegradation [33,39]. The foundational two mechanisms of biodegradation are metabolic degradation and co-metabolism [39]. Tang et al. [44] proposed a demonstration for the biodegradation of paracetamol within *Chlorella subellipsoidea* and investigated the impact of paracetamol on algal growth. The results revealed that paracetamol improved the microalgal growth. However, it reduced the biomass increment as the concentration of paracetamol increased. Antibiotic biodegradation via *Chlorella vulgaris* was demonstrated by Kiki et al. [45]. The study claimed an enhanced antibiotic removal upon utilizing a mixed antibiotic culture, producing lower toxicity transformed products.

3. *Chlorella* microalgae for wastewater phycoremediation

3.1. *Chlorella vulgaris*

Chlorella vulgaris is a microalgal species capable of sustainably treating distinct pollutants from various kinds of wastewater. In one study, *chlorella vulgaris* performed best among other types of microalgae in bioremediating nutrients like TN, TP, COD, and BOD_5 from raw aquaculture effluent in a semi-continuous remediation system of three independent sequential containers. The capacity of three sequential reservoirs to remediate nutrients was better than that of one reservoir with the same volume [46]. Fast biosorption capacity of Cd could be achieved by *Chlorella vulgaris* after 8 h of exposure to wastewater, revealing carboxylic moieties of the cell walls to be vital in Cd removal [47]. The synergistic effects of coupling *Chlorella vulgaris* biosorption potentialities and the photocatalytic capabilities of TiO_2 nanoparticles (NPs) to develop a bio-nanohybrid catalyst push algal remediating technologies towards improved metal biosorption and photocatalytic activities. The surface interactions between *Chlorella vulgaris* and TiO_2 are pivotal in stimulating the functional properties [48].

Among other microalgal species, *Chlorella vulgaris* was the fastest and most efficient in removing 19 different kinds of pharmaceuticals from wastewater in 12 days while simultaneously accumulating only a small amount of such compounds in their biomass (<2 %), allowing for further reuse. The complete removal of Trihexyphenidyl, Clomipramine, Flecainide, Orphenadrine, and Memantine and the failure to detect them in the algal biomass offers *Chlorella vulgaris* a detoxifying mechanism that degrades non-polar pharmaceuticals to less toxic compounds [36]. *Chlorella vulgaris* monoculture and microalgal-bacteria consortium mediums achieved adequately similar removal rates. However, the monoculture was faster in removing different pharmaceuticals [49]. Waste microalgae are still a valid option as environmentally acceptable adsorbents for the extraction of antibiotics from aqueous streams because of their viable adsorption potentials. Ciprofloxacin adsorption on waste *Chlorella vulgaris* is sensitively affected by alerts in contact time, pH, and adsorbent dose impact [50]. Both living and non-living *Chlorella vulgaris* possess great potential to remove low concentrations of fluoxetine from domestic wastewater. Nevertheless, living *Chlorella vulgaris* has a higher biosorption capacity than non-living ones, as it can simultaneously remove fluoxetine and nutrients from wastewater in one step [51].

Synthesized *Chlorella vulgaris*-mediated green NPs of an average size of 32.2 nm and hydrodynamic size of 52.5 nm were utilized to remediate azo dye-polluted wastewater and for antibacterial activity purposes. The NPs exhibited a dye removal efficiency of 94 % and excellent reusability, retaining 80 % activity after 5 cycles [52]. *Chlorella vulgaris* can also be employed for hybrid bio-photocatalysts. Preparing a hybrid bio-photocatalyst from *Chlorella vulgaris* and ZnO to remediate oily wastewater treatment achieved superior 100 % hydrocarbon content biodegradation from low-concentrated oily wastewater. The hybrid system's performance is inversely proportional to the initial hydrocarbon concentration as it drops when the concentration increases [53]. Another suggested solution for oily wastewater treatment via microalgae is the construction of a microalgae-bacteria consortium system made of *Chlorella vulgaris* and *Rhodococcus erythropolis*. While maintaining the same conditions, the addition of terminal electron acceptors plays a pivotal role in facilitating the biodegradation of waste motor oil. Fe^{3+}electron acceptor achieved up to 99.2 % oil removal efficiency, and SO_4^{2-} achieved 97.1 % [54].

The consortium of *Chlorella vulgaris* and activated sludge resulted in a greater than 50 % boost in the removal efficiency of distinct pathogens compared to using activated sludge solely. The effect of CO_2 or O_2 addition or photoperiod on the performance of *Chlorella vulgaris* for pathogen removal depends on the pathogenic bacteria inactivation mechanism [55]. Introducing commercial biochar to a consortium of *Chlorella vulgaris* and activated sludge photobioreactor increases the removal of some pesticides like chlorpyrifos and cypermethrin compared to a similar system with the consortium only. Biochar addition boosts microorganism immobilization, which overcomes the high-cost issues for the recovery of microalgae and biomass from treated water usually associated with algal/bacterial consortiums [56]. Abdel-Razek et al. [57] confirms the superior impact of microalgae consortium systems by modifying a system containing *Chlorella vulgaris, Scenedesmus quadricuda*, and *Spirulina platensis*, resulting in enhanced removal of organophosphate pesticide from urban wastewater with a removal efficiency of up to 99 %.

3.2. Chlorella sp

Consortiums of *Chlorella* sp. with other types of microalgae or bacteria have elevated the removal of several nutrients from different types of wastewater. A microalgal consortium comprising *Chlorella* sp. and *Scenedesmus* sp. possesses significant potential for nutrient removal up to 98 % [58]. One other study demonstrated the critical role of the synergy impact of *Chlorella* sp. and activated sludge for tolerating the high concentrations of ammonia nitrogen and nutrient removal from wastewater, shedding light on optimizing the C/N ratio given the fact that a suitable C/N ratio facilitates the metabolism and ability of microalgae to absorb nutrients (N and P) from wastewater [59]. Other wastewater properties like TOC/TN ratio impact microalgae growth, hence, nutrient removal. Increasing wastewater's TOC/TN ratio gradually increases the growth of *Chlorella* sp. and nutrient removal, where at a ratio of 24, >99 % of total nitrogen and phosphorous could be removed [60].

Chlorella sp. is known to purify swine wastewater efficiently. Zn and Mn can be effectively removed from swine wastewater using living *Chlorella* sp. However, it has been found that the participation of Mn boosts the removal of Zn, in contrast with the inhibited removal of Mn in the presence of Zn [61]. Considering the superior performance of *Chlorella* sp. for heavy metal removal, it performs better in removing distinct heavy metals from textile wastewater than *Chlorella sorokiniana*. Although both kinds manifested 100 % removal of Pb, the removal of V was hindered in the presence of *Chlorella sorokiniana*, as it was about 40–60 %, while *Chlorella* sp. exhibited 100 % removal [62].

Chlorella sp. efficiently degrades distinct veterinary antibiotics from swine wastewater, removing up to 100 % tetracycline [63]. Besides, *Chlorella* sp. biomass could be utilized as a biocatalyst and an organic alternative for chemical oxidants. Its combination with Fe^{2+} ions and ultraviolet (UV) light enhanced the remediation of ciprofloxacin, a veterinary pharmaceutical, from wastewater up to 99 % [64]. Combining biogenic Mn oxides (Bio-MnOx) and Mn-oxide Microalgae (MnOMs) to remove pharmaceuticals from wastewater is more cost-effective than using Bio-MnOx produced by fungi or bacteria. This synergistic mechanism with MnOMs containing *Chlorella* sp. along with bio-MnOx notably increased the removal efficiency of carbamazepine and diclofenac pharmaceuticals from wastewater [65,66]. Synthesizing wet-torrefied *Chlorella* sp. microalgal biochar is recognized as a waste-derived and low-cost adsorbent for the removal of cationic and anionic dyes from wastewater, with maximum adsorption capacities of 113 mg/g and 164.35 mg/g for methylene blue and Congo red dyes, respectively [67]. Other studies considered a microalgal system with *Chlorella* sp. as a tertiary step for dye wastewater treatment. Complementing fungal/bacterial consortium with further treatment using *Chlorella* sp. achieved final removal rates of color units of 91 % [68].

Outdoor pilot-scale *Chlorella* sp. microalgae-based tubular photobioreactor designed to eliminate propanil and acetamiprid pesticides from wastewater efficiently. The photobioreactor with modified Mann and Myers medium and 8 days hydraulic and solid retention time possessed a superior biodegradation ability of propanil with a 99 % efficiency and 71 % removal efficiency of acetamiprid pesticides [69]. *Chlorella* sp. proved superior performance in the removal of other pesticides as Hu et al. [70] showed that 83 % of atrazine was removed from the catalytic degradation solution using the *Chlorella* sp. biodegradation mechanism. Among other kinds of microalgae and cyanobacteria, *Chlorella* sp. achieved the highest Chlorpyrifos removal efficiency from agricultural wastewater [71].

3.3. Chlorella sorokiniana

The application of *Chlorella sorokiniana* in wastewater treatment has gained interest due to its rapid growth rate, high biomass yield, and ability to remediate different pollutants. *Chlorella sorokiniana* possessed great potential in removing nutrients and COD during its cultivation in raw dairy wastewater with moderate and high COD levels while obtaining high biomass concentration. The increase in inoculum size, up to a certain limit, had a significant effect on the microalgae's biomass production and remediation performance. In addition, it increases its stability to withstand the harsh conditions present in wastewater [72]. The cultivation of *Chlorella sorokiniana* in a hexagonal airlift flat plate photobioreactor of improved hydrodynamic parameters showed a 61 % enhancement in microalgae growth compared to traditional photobioreactors. It also showed superior and accelerated removal ability of different nutrients from wastewater [73]. Benefiting from *sorokiniana*'s potential to assimilate N and P and indigenous DMW bacteria's ability in organics removal, Chang et al. [74] developed a microalgae-bacteria consortium to treat dairy manure wastewater. It achieved 90.2 %, 100 %, 84.3 %, and 97.8 % efficacy for the removal of TN, TP, COD, and BOD, respectively.

Chlorella sorokiniana performed better than other microalgae species in removing nutrients and Cu from alginate-pretreated real industrial wastewater. It also possessed higher biomass production, tolerance to toxicity, and protein content [75]. Complete biodegradation of oseltamivir antiviral drugs from synthetic municipal wastewater was obtained using *sorokiniana* for the 10 mg/L concentrated sample. However, it was spotted that biomass growth was inhibited at high concentrations of the contaminant [76]. Some studies combined the use of microalgae with other materials to enhance their performance in heavy metals removal from wastewater. For instance, Lapeñas et al. [77] coupled the utilization of biotechnology and nanotechnology by adding manganese-doped ferrite NPs to the microalgal suspension. The modified cells with the magnetic NPs achieved an enhanced ability to sequester Cr, Co, and Ni and adsorption capacity without affecting the microalgae's growth. It indicates that the combination of biosorption and chemisorption can synergistically improve the remediation of metal-contaminated water. Politaeva et al. [78] prepared an adsorbent using the residual biomass of *Chlorella sorokiniana*. The addition of graphite and chitosan led to the formation of a porous and layered structure, which enhanced the biosorption capacity of the adsorbent towards Pb and Cu with high purification efficiency.

Among distinct microalgal strains tested for azo dye-contaminated industrial wastewater decolorization, *Chlorella sorokiniana* exhibited the best performance. The bioaccumulation of the dye in the microalgal cells was measured, and the system achieved the highest decolorization efficiency [40]. The use of green synthesized Ag NPs made of microalgae extracts as an alternative to physiochemical synthesized toxic NPs showed great potential in the removal of several dyes. It possessed a photocatalytic degradation efficiency of 97.0 %, 95.7 %, 94.9 %, and 56.0 % for crystal violet, methylene blue, eosin Y, and rhodamine B dyes, respectively [79]. Furthermore, *Chlorella sorokiniana* and *Aspergillus* sp. fungus consortium have a great decolorization rate and Disperse Red 3B degradation from wastewater. The consortium possessed good stability and adaptation to wastewater containing high concentrations of dye and salt [80]. A higher removal of the phenolic component hydroxytyrosol from oil mill wastewater with enhanced antioxidant activity was obtained by *Chlorella sorokiniana* compared to *Scenedesmus quadricauda* [81]. Moreover, its great ability to remove pathogens from anaerobically-digested black wastewater was also observed [82].

3.4. Chlorella pyrenoidosa

Several studies tested the reduction of nutrients using *Chlorella pyrenoidosa* during its cultivation and by coupling it with other systems or cultures. For instance, the cultivation of *Chlorella pyrenoidosa* in wastewater under mixotrophic conditions almost led to the complete elimination of ammonium and orthophosphate [83]. Outstanding nutrient removal rates were obtained while cultivating it in a seawater-wastewater mixture. The microalgae possessed great adaption to seawater stress by promoting the antioxidant enzyme content [84]. Co-cultivating *Chlorella pyrenoidosa* with fungi can effectively enhance its wastewater purification ability from COD, TN, and TP and its biomass yield compared to individual cultures. Moreover, the encapsulation of the microalgae in fungal pellets eases the biomass harvesting process [85]. In addition, it showed great potential for nutrient recovery from biogas slurry when co-cultured with other microalgae strains [86]. The rapid growth of *Chlorella pyrenoidosa* was achieved once cultivated inside a membrane photobioreactor (MPBR) containing hydrolyzed wastewater produced from a membrane bioreactor (MBR). The hybrid MBR-MPBR system obtained an excellent reduction of 96.7 %, 98.0 %, and 95.9 % for TN, TP, and TOC, respectively [87].

The outdoor cultivation of *Chlorella pyrenoidosa* in oilfield-generated wastewater demonstrated its capability to remediate different toxic heavy metals and nutrients simultaneously. The biosorption ability of *pyrenoidosa* towards heavy metals results from the tendency of metal ions to bond to the polyelectrolytes found within the microalgae's cell walls [88]. Ran et al. [89] tested the response of several green microalgae species to Cd and Pb, where *Chlorella pyrenoidosa* possessed the highest tolerance when exposed to high concentrations of these heavy metals. It experienced less damage to the microalgal cells' surface and interior due to its higher glutathione content and peroxidase activity compared to the other species. *Chlorella pyrenoidosa*-derived biochar was produced by Rohman et al. [90] via pyrolysis and showed non-porous characteristics. However, once modified with sodium bicarbonate, it possessed significantly enhanced material properties and characteristics with high adsorption capacity towards heavy metals.

Chlorella pyrenoidosa demonstrated a notable decrease in the concentration of tetracycline antibiotic in wastewater, with 93.9 % removal efficiency within 2 days and reaching up to 99 % on day 11 for the low-concentrated wastewater (10 mg/L). However, with the increase of the pollutant's initial concentration in the wastewater, the removal ability of the microalgae deteriorates. The degradation of tetracycline produced different transformation products that show no significant toxicity [91]. Wang et al. [92] investigated the different mechanisms and pathways for the elimination of sulfadiazine using *pyrenoidosa*, where biodegradation was the main mechanism, followed by photodegradation. The degradation products can undergo several reactions that minimize harmful environmental effects, including hydroxylation, oxidation, ring opening, and bond breakage. Furthermore, this microalgae species showed superior performance in the removal of pathogenic bacteria from real sewage wastewater. It was concluded that photooxidation governs the interaction between *Chlorella pyrenoidosa* and *E. coli*, as an increase in light intensity reduced removal time by 93 % [93]. In the decolorization of real dye industrial wastewater, immobilized cells of *pyrenoidosa* were employed as an adsorbent and achieved a maximum of 98 % efficacy [94]. The application and results of some of the discussed studies are listed in Table 2.

4. *Chlorella* microalgae biorefinery for resource recovery

Sustainably transforming and converting renewable sources into beneficial products defines the concept of circular bioeconomy. Closed-loop biorefineries integrated with wastewater result in several environmentally favorable advantages, like valuable resource recovery and reduced ecological footprint [95]. Microalgal technologies possess the potential as a promising and renewable resource in biorefineries due to their flexible and eco-friendly characteristics like photosynthetic activity, CO_2 capture, and accelerated growth. Distinct types of wastewaters include various metabolites like protein, lipids, pigments, antioxidants, and carbohydrates that could be utilized in producing biofuels, biofertilizers, food compounds, enzymes, and many other esteemed bioproducts. Additionally, employing microalgae in wastewater treatment opens up an additional opportunity for recycling and reusing water, particularly for industrial or agricultural purposes [95,96]. The vast amounts of microalgal suspension produced upon cultivation necessitate specific methods to harvest economically and technically viable biomass. Such methods or their combinations are pivotal for producing the final valuable bioproducts by concentrating the algal suspensions or biofilms [97]. Algal-based biofuels comprise merits that make them attractive when compared to fossil fuels. For instance, they are biodegradable, readily available in nature, renewable, produce less emissions, and contribute towards sequestrating CO_2. Autotrophic organisms produce biofuels by converting solar energy into chemical energy through photosynthesis, hence, algae transform CO_2 and light into biomass [98]. The main bioproducts derived from microalgal biomass include biodiesel, biohydrogen, bioethanol, biogas (methane and CO_2), and biofertilizers (Table 3). The conversion process of each microalgae-derived bioproduct is shown in Fig. 4.

Table 2
Wastewater bioremediation of pollutants using Chlorella microalgae.

Microalgae species	Targeted pollutants	Mechanism	Wastewater source	Removal efficiency	Ref.
Chlorella vulgaris	TN, TP, COD	–	Aquaculture wastewater	100 %, 100 %, >96 %	[46]
Chlorella vulgaris	Ciprofloxacin	Biosorption	Real hospital wastewater	100 %	[50]
Chlorella vulgaris	Clomipramine, trihexyphenidyl, flecainide, orphenadrine, memantine, biperiden, bupropion, diphenhydramine, hydroxyzine	Biosorption, bioaccumulation, biodegradation	Synthetic wastewater	100 %, 100 %, 100 %, 100 %, 100 %, 93 %, 82 %, 98 %, 93 %	[36]
Chlorella vulgaris	Motor oil	Biodegradation	Synthetic wastewater	99.2 %	[54]
Chlorella vulgaris	Chlorpyrifos, cypermethrin pestecides	Biosorption and biodegradation	Synthetic wastewater	88.8 %, 93.1 %	[56]
Chlorella sp. G-*9*	TN, TP, TOC	Biodegradation	Wastewater treatment plant	99.61 %, 99.79 %, 93.1 %	[60]
Chlorella sp. *HL*	Zn, Mn	Biosorption and bioaccumulation	Swine wastewater	97.2 %, 42.7 %	[61]
Chlorella sp.	Tetracycline, chlortetracycline, doxycycline, oxytetracycline	Biosorption	Swine wastewater	100 %, 100 %, 91 %, 83 %	[63]
Chlorella sp.	Malachite Green dye	Biosorption	Non-domestic wastewater	91 %	[68]
Chlorella sp.	Propanil, acetamiprid pesticides	Biodegradation	Synthetic wastewater	99 %, 71 %	[69]
Chlorella sorokiniana Pa.*91*	NH_3, NO_3^-, PO_4^{3-}, COD	–	Municipal wastewater	91 %, 99 %, 97 %, 93 %	[73]
Chlorella sorokiniana	Cu, Pb	Biosorption	Industrial wastewater	> 90 %	[78]
Chlorella sorokiniana	Oseltamivir	Biosorption, biodegradation	Synthetic municipal wastewater	100 %	[76]
Chlorella sorokiniana	Hydroxytyrosol	Bioaccumulation	Real OMW	69 %	[81]
Chlorella sorokiniana	Crystal violet, methylene blue, eosin Y, rhodamine B dyes	Biosorption and biodegradation	Synthetic wastewater	97.04 %, 95.75 %, 94.90 %, 56.05 %	[79]
Chlorella pyrenoidosa FACHB-9	COD, TN, TP	Biosorption	Starch wastewater	92.1 %, 83.6 %, 96.6 %	[85]
Chlorella pyrenoidosa	Fe, Cu, Pb, Cd	Biosorption and bioaccumulation	Oilfield wastewater	76.74 %, 73.39 %, 72.86 %, 48.42 %	[88]
Chlorella pyrenoidosa	Tetracycline	Biosorption, bioaccumulation, biodegradation	Synthetic wastewater	99 %	[91]
Chlorella pyrenoidosa	TN, TP, TOC	Biosorption	Municipal wastewater	96.7 %, 98.0 %, 95.9 %	[87]
Chlorella pyrenoidosa	*Escherichia coli*, total bacterial count, *Enterobacteriaceae, Salmonella* sp.	Photooxidation	High-strength synthetic municipal wastewater and real sewage wastewater	99.9 %, 92 %, 98 %, 96 %	[93]

Table 3
Chlorella microalgae biorefinery and process characteristics.

Microalgae species	Conversion process	Produced bioproduct	Main cultivation/process characteristics	Highlights and findings	Ref.
Chlorella sorokiniana	Transesterification	Biodiesel	Diluting livestock wastewater with domestic wastewater enhances biodiesel production	Great potential for biodiesel production while treating wastewater	[101]
Chlorella vulgaris	Transesterification	Biodiesel	Undiluted textile wastewater used for cultivation	Efficient biodiesel production from undiluted textile wastewater	[102]
Chlorella pyrenoidosa	Dark fermentation	Biohydrogen	Batch mode process for biohydrogen production	Generation of 45.5 mL H_2/g VS	[105]
Chlorella vulgaris	Photofermentation and biophotolysis	Biohydrogen	Exogenous carbon substrates (e.g., glucose, mannitol, sorbitol) enhance production	1246 mL/L biohydrogen using 10 g/L glucose and 1:1.5 v/v inoculum	[106]
Chlorella vulgaris	Fermentation	Bioethanol	Adjusted inoculum size, light intensity, and harvesting period	84.2 % bioethanol yield with 4.2 g/L concentration	[109]
Chlorella vulgaris	Fermentation	Bioethanol	Cultivated in oil industry wastewater	Bioethanol production of 18.2 mL/100 g of biomass	[110]
Chlorella vulgaris	Anaerobic digestion	Biogas (Methane)	Heat pretreatment boosted digestibility and methane yield by 83 %	Methane yield of 408 mL CH_4/g VS	[114]
Chlorella vulgaris	Anaerobic digestion	Biogas (Methane)	Thermal pretreatment at 121 °C for 0.3 h resulted in significant yield enhancement	322 mL CH_4/g VS with 108 % enhancement compared to untreated	[115]
Chlorella minutissima	–	Biofertilizer	Cultivation in sewage wastewater	Enhanced economic yield of spinach and baby corn	[118]

Through transesterification, lipids accumulate in microalgal cells and are separated and converted into biodiesel, which is a process related to chemical conversion [99]. The transesterification reaction is achieved when a short chain of alcohol (commonly methanol) reacts with lipids, especially triacylglycerides (TAG), in the existence of a catalyst to generate glycerol and methyl esters of fatty acids (FAME). Later, it contributes to the production of biodiesel as an environmentally friendly fuel that inhibits the emission of toxic gasses [100]. Cultivating microalgae in mixed wastewater is adopted as an alternative to conventional dilution with fresh water, which enhances biodiesel production. Diluting livestock wastewater with domestic wastewater for cultivating *Chlorella sorokiniana* JD1-1 possesses great potential for

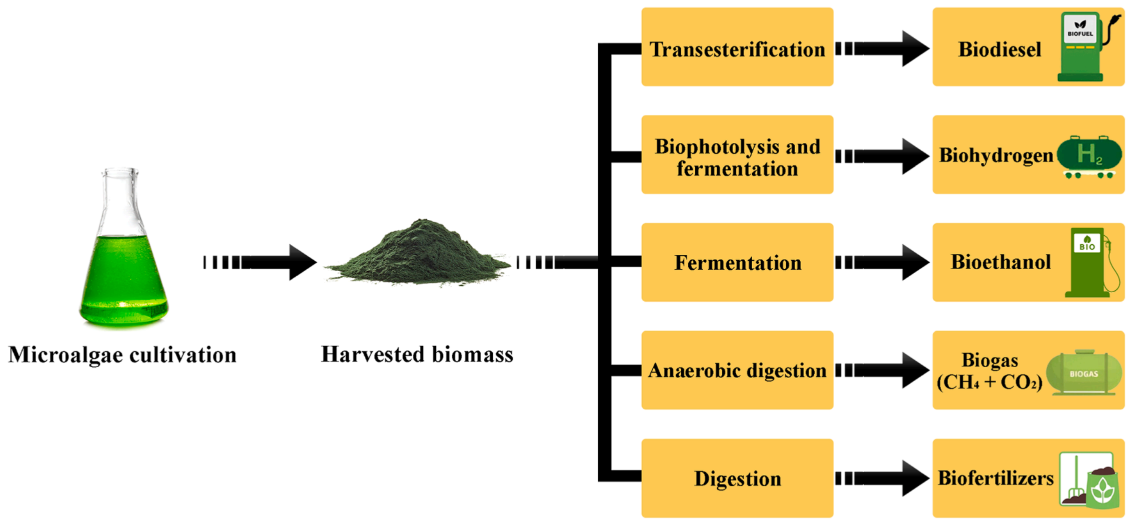

Fig. 4. Microalgae-derived bioproducts and their conversion process.

biodiesel production while simultaneously treating wastewater in a cost-effective and sustainable strategy [101]. Although diluting textile wastewater with fresh water yielded higher biodiesel production, the enhancement percentage is insignificant compared to undiluted wastewater. This concludes the robustness of *Chlorella vulgaris* in treating undiluted textile wastewater for efficient biodiesel production [102].

Biohydrogen is recognized as a promising fuel source because of its elevated calorific value and clean oxidation properties [103]. When it is produced by microalgae, it is classified into light-dependent and light-independent pathways. The light-dependent pathway includes biophotolysis and photofermentation. Meanwhile, the light-independent pathway includes dark fermentation or heterotrophic fermentation [104]. *Chlorella pyrenoidosa*'s capability to produce biohydrogen via dark fermentation in batch mode process suppressed other kinds of microalgae (*Scenedesmus obliquus* and *Chlorella sorokiniana*), achieving up to 45.5 mLH_2/g VS [105]. Since exogenous carbon substrates are crucial for elevating the fermentative biohydrogen production, boosting glucose and its derivatives, including mannitol and sorbitol, positively enhances the growth of microalgae in a co-culture system of wastewater and activated sludge. Among its derivatives, 10 g/L of glucose with 1:1.5 v/v inoculum, the highest biohydrogen production was observed at 1246 mL/L using *Chlorella vulgaris* compared to the control medium with only maximum biohydrogen production of 5.6 mL/L [106].

Bioethanol is produced through the chemical or enzymatic breakdown of polysaccharides (mannans, xylans, cellulose, and sulfated glycans) existing in some microalgae cell walls into simple sugars that can be converted into bioethanol. Microalgae like *Chlorella, Scenedesmus, Porphyridium, Dunaliella, Spirulina*, and *Chlamydomonas* comprise relatively elevated content of carbohydrates reaching up to 50 % of the dry weight of starch, cellulose and glycogen, which are essential constituent materials for bioethanol production. Bioethanol obtained from algal sources is produced through microalgal photosynthesis and anaerobic fermentation [107]. Additionally, because of their high fermentable sugar content (low lignin) and facilitated saccharification, microalgal-based carbohydrates are a more viable and eco-friendlier alternative for efficient bioethanol production [108]. Adjusting several factors, including inoculum size, light intensity, and harvesting period, improves *Chlorella vulgaris* performance and obtains biomass productivity, carbohydrate content, and carbohydrate concentration of 0.49 g/L/day, 49.3 %, and 1.8 g/L, respectively. Furthermore, it promotes successful ethanol fermentation with bioethanol yield and concentration of 84.2 % and 4.2 g/L [109]. Similarly, *Chlorella vulgaris* efficiently produced bioethanol by cultivating it in oil industry wastewater, producing 18.2 mL/100 g of biomass [110].

Microalgae-based biogas is an alternative sustainable source of energy. Algal biomass compounds, including protein, sugar, and lipid, are converted into methane and CO_2 via anaerobic digestion containing a synergistic microbial population. The produced biomethane can be utilized as a fuel or chemical feedstock [111]. Two crucial factors affecting the methane yield in anaerobic digestion, including the microalgae cell, were composition and its participation in the total cell mass. The high protein content in various microalgae species leads to elevated ammonia-nitrogen content during anaerobic digestion. Since ammonia possesses a high potential to permeate through the cell walls of microalgae, this influences methane yield because of ammonia inhibition. The impact of inert organic matter on methane yield is believed to be stronger than that of energy-rich macromolecules, as elevated lipid content is not usually associated with elevated methane yield [112]. CO_2 produced associated with methane through anaerobic digestion is utilized as a carbon source for algae growth [113]. *Chlorella vulgaris* is an excellent microalgae strain for the production of methane with the aid of domestic wastewater. Heat pretreatment proved to be the most competent method to boost the anaerobic digestibility and enhance the methane yield by 83 % to achieve 408 mL CH_4/g VS [114]. In a similar study, the impact of high and low thermal pretreatment was studied to enhance the biomethane yield of *Chlorella vulgaris*. Thermal pretreatment with conditions of 121 ˚C for 0.3 h resulted in the greatest methane yield (322 mL/g VS), which equals 108 % enhancement compared to the untreated situation [115].

Biofertilizers are employed to improve plant production by incorporating organic matter and nutrients into the soil, typically applied in the form of biomass. They can be obtained in distinct ways, namely, microalgal extracts, dry biomass production, and digestion of microalgal biomass [116]. Using microalgae biomass for the development of biofertilizers brought multiple advantages to the agricultural fields. Utilizing microalgal biomass for agricultural purposes enhanced nutrient absorption, biomass accumulation, and germination index. Additionally, microalgal biomass derived from wastewater can be considered a nutrient source for agricultural applications [117]. Higher enzymatic activity of dehydrogenase, urease, and nitrate could be achieved when adding microalgal-based fertilizer, which indicates an improvement in crop development. As a result, the incorporation of 100 % N algal-based biofertilizer derived from *Chlorella minutissima* cultivated in sewage wastewater promoted the economic yield of spinach and baby corn [118].

5. Application of AI and ML algorithms

The swift advancement and development of AI in the past few years has resulted in its integration into a wide range of applications. Combining AI-driven data analysis and its optimization capabilities with microalgae-based treatment systems can enhance the removal efficiency of contaminants from wastewater and the generation of valuable biomass by optimizing the operational parameters. This can reduce the pressure on conventional agricultural resources, reduce costs, promote sustainable bioproduction, and contribute to the bioeconomy. In addition, AI and ML algorithm approaches can adequately monitor, forecast uncertainty, and identify faults within complex environmental systems [20]. The different applications of AI and ML algorithms in microalgae processes are illustrated in Fig. 5.

5.1. AI and ML in microalgal wastewater treatment

AI-based models and predictive analytics are crucial in optimizing the overall microalgae cultivation and wastewater treatment processes. These models can forecast microalgae growth rates, biomass composition and production, and the removal of specific pollutants. The initial essential stage in the development of microalgae-related products involves the optimization of resource input to achieve a high biomass yield of consistent quality. AI and ML algorithms can process vast amounts of data and adjust several parameters that affect microalgae productivity, including temperature, pH, light exposure, aeration, and wastewater and medium characteristics such as COD and BOD levels and nutrient concentrations [119]. These parameters are greatly reliant and specific to the microalgae species utilized. Therefore, it is essential to optimize growth conditions that best suit the strain used to obtain the highest biomass yield while possessing the best treatment efficiency.

Several studies employed AI and ML models that predict biomass productivity and treatment performance of *Chlorella* and suggest optimized initial and operational parameters. The characteristics of treated wastewater effluent by *Chlorella vulgaris* were predicted using artificial neural network (ANN). The input data to the network, including the initial concentrations of PO_4^{3-} and NH_4^+, light intensity, and initial biomass, were set to predict the final concentrations of the ions in the effluent. The obtained network model showed a high correlation between the simulated and experimental results with $R^2 > 0.95$ [120]. Multilayer feedforward ANN achieved $R^2 > 0.99$ and a low mean squared error (MSE) of <0.1 in optimizing the performance of *Chlorella vulgaris* in photobioreactors for the removal of several contaminants from wastewater [121]. Ansari et al. [122] predicted the biomass growth and algal dry cell weight of microalgae cultivated in a nutrient-supplemented secondary treated wastewater via three-layer feedforward backpropagation ANN. Temperature, pH, TN, TP, dissolved O_2, and electric conductivity were set as inputs, and the

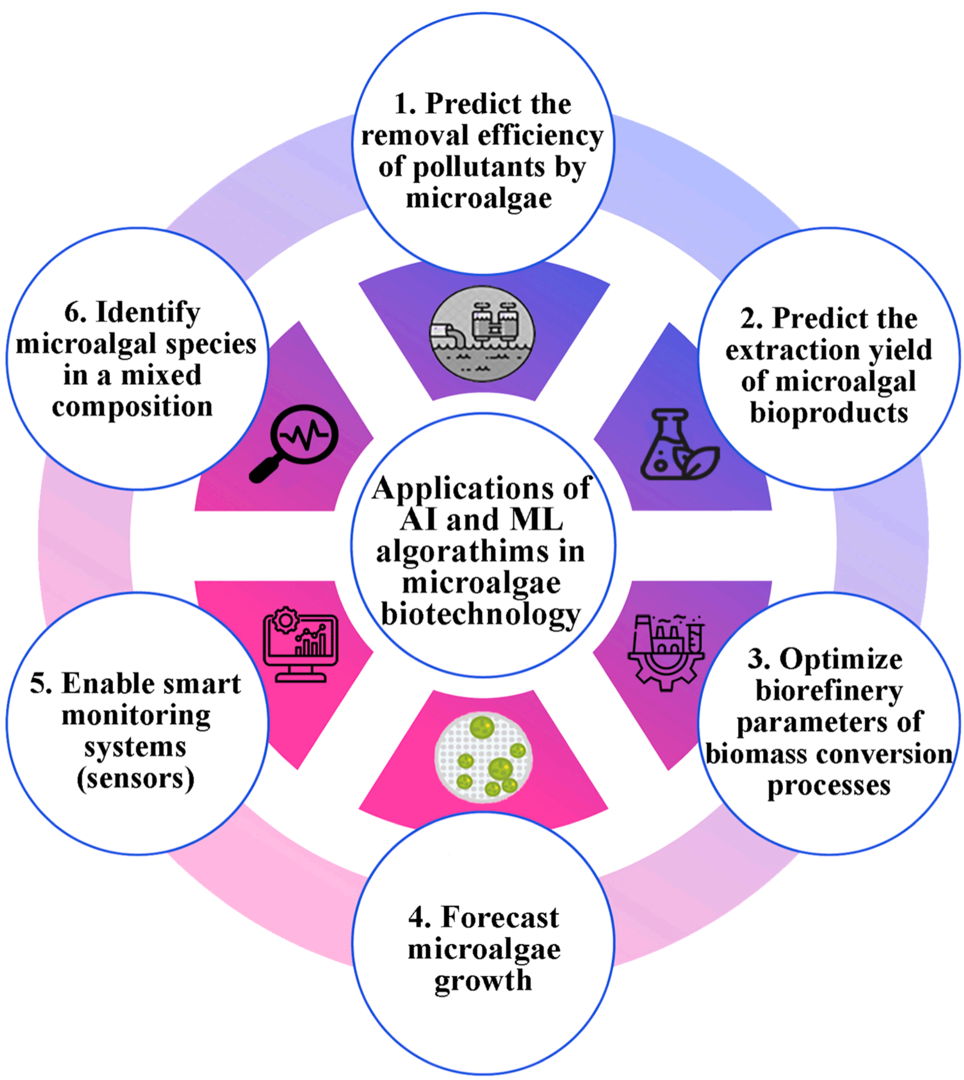

Fig. 5. Applications of AI and ML algorithms in microalgae processes.

developed ANN model achieved high prediction accuracy with $R^2 \sim$ 0.98.

The presence of several nutrients in the growth culture medium can be optimized by investigating their effect on the properties of the produced microalgae biomass using feedforward ANN [123]. Podder & Majumder [124] developed an ANN model to predict the performance of *Chlorella pyrenoidosa* in the removal of As(III) and As(V) heavy metals from wastewater. The influence of the initial pollutant concentration, pH, inoculum size, and contact time was considered. The model showed a superior prediction accuracy for the phycoremediation of both ions from the wastewater, with a high correlation coefficient of R^2 ~0.999. Lavrinovičs et al. [125] used xT SAAM, an industrial AI-based platform, to optimize initial conditions using data from batch experiment series. The optimization model outcomes proposed the combination of one-day biomass P-starvation with low initial biomass concentration, which led to an elevation in the contaminant removal efficiency of *Chlorella vulgaris* from wastewater by 101.7 %. The possibility of recycling the culture medium and its influence on the growth of *Chlorella vulgaris* in semi-batch cultivation can be tested using an AI-enabled Internet of Things (IoT) framework [126].

5.2. AI and ML in microalgal bioproduction

Various processes have been employed to generate green and eco-friendly products to replace conventional toxic and harmful ones. However, expensive and time-consuming tests required to be conducted to develop and enhance these processes make it challenging to deal with [127]. The application of AI and ML in the conversion of biomass to produce bioproducts has emerged in recent years. They can optimize several input parameters of different biomass conversion processes, such as microalgae compositions and content, temperature, reaction time, catalyst loading, and energy consumption [128,129]. The optimal conditions of the direct transesterification of *Chlorella pyrenoidosa* for biodiesel production were investigated using multilayer ANN. The significance of each of the several influencing parameters, including temperature, reaction time, concentration of catalyst, and solid-biomass ratio, was evaluated [130]. Similarly, ANN, response surface methodology (RSM), and genetic algorithm (GA) were employed to optimize the conditions of the supercritical methanol transesterification process to produce FAME from *Chlorella CG12*. The ANN model, coupled with the GA-obtained optimized conditions, predicted a 99.2 % conversion efficiency compared to the 98.1 % efficacy achieved by the experiment, which performed slightly better than RSM [131].

Two ML algorithms, gradient boosting regression (GBR) and random forest (RF), were utilized to predict and optimize hydrothermal liquefaction conditions for biofuel production from *Chlorella*. The results obtained from GBR exhibited better performance for both single and multi-target task prediction than RF [132]. Teng et al. [133] assessed the use of *Chlorella vulgaris* for biofuel production and predicted its optimal thermal conversion conditions using a neuro-evolutionary approach. The Progressive Depth Swarm-Evolution (PDSE) algorithm was utilized to model data of catalytic thermal degradation of the microalgae obtained from thermogravimetric analyzes (TGA). At the suggested operational conditions and with HZSM-5 zeolite as a catalyst, they obtained a high biomass conversion of 88.3 % from the *Chlorella*. Furthermore, deep neural network was employed to investigate the potential of several *Chlorella* species for biofuel production via pyrolysis. The TGA temperature and heating rate data set were considered input variables to develop the model, and the obtained model showed a high regression coefficient ($R^2 \sim 0.997$) and a low MSE of 10^{-6} [134].

The Bayesian regularization ANN algorithm was utilized to enhance the production of green hydrogen from *Chlorella vulgaris* biomass via formic acid-mediated hydrothermal carbonization [135]. Sobri et al. [136] used a ML approach to derive a kinetic model that predicts hydrogen production from *Chlorella vulgaris* biomass by conversion from pre-treated palm kernel expeller waste. The thermal pre-treatment duration was an input to their model. Kumar et al. [137] employed ANN and RSM to study the valorization of *Chlorella sorokiniana* biomass residual to generate biofuel and value-added chemicals via pyrolysis. Both models possessed very high prediction ability with accurate predictions. However, the model obtained from ANN demonstrated a slightly higher prediction accuracy than RSM, with $R^2 \sim 0.998$ compared to 0.99. A summary of the work conducted in these studies is listed in Table 4.

6. Challenges and future outlook

6.1. Challenges and economic sustainability

The application of *Chlorella* as a sustainable method for wastewater treatment proved its potential due to its various advantages, including its excellent bioremediation ability of several contaminants. However, most of the studies mentioned in this review were performed on a laboratory scale, while a few others were on a pilot scale, and the research does not go beyond that. The implementation of microalgae-based wastewater treatment for large-scale operations is still a difficulty, mainly because of the high production and harvesting costs of biomass production [138]. Algal biomass production costs about 290 to 587 € per 1 kg of dry-weight biomass, which depends on the utilized rector type and cultivation conditions [139]. In addition, real industrial wastewater comes with varying pollutant concentrations and characteristics, where the microalgae will be exposed to harsher environmental and seasonal conditions. This will highly affect its treatment performance by reducing bioremediation efficacy and biomass yield. For instance, the presence of some contaminations, such as *Herbivorous protozoa* and *zooplankton*, can severely harm microalgae during cultivation as they feed on organic matter. They can significantly reduce algal concentrations in open reactors, with up to 90 % algal biomass reduction within a few days [32]. Although contamination can be prevented using closed photobioreactors on a laboratory scale, large-scale photobioreactors' high installation and operational costs limit their application [140].

Each microalgal strain possesses its ideal growth conditions for the different types of microalgae and their different species for each type [119]. Their growth is impacted by several factors, including nutrient presence, temperature, illumination and light intensity, pH, CO_2 concentration, and other wastewater characteristics. The effect of light intensity and temperature on the production of biomass, lutein, and lipids from *Chlorella sorokiniana* was studied by Patel et al. [141]. The biomass and lutein yield has increased with the light intensity increase from 5000 Lux to 10,000 Lux. However, a further rise in the intensity showed reduced biomass productivity. Whereas lipid accumulation was found to rise with the rise of light intensity. Similarly, the biomass produced increased initially with the increase in temperature and then dropped after exceeding a specific value. Among the challenges that affect microalgae growth while dealing with real industrial wastewater is its high turbidity. High turbidity can inhibit growth by limiting light availability, by which the light penetration into the medium will be reduced [142]. This reduction adversely impacts the photosynthetic rate, specifically for submerged microalgae that are unable to float or reach the surface [143]. Nevertheless, this can be solved by pretreating the wastewater via flocculation, for instance, to remove the suspended solids that cause turbidity before introducing the microalgae.

One of the most serious challenges that *Chlorella* microalgae-based wastewater treatment faces is the efficiency of microalgae biomass recovery, especially in suspended cultivation. Harvesting the microalga biomass after wastewater bioremediation is considered the most critical concern, for being expensive and yet to be optimized for large-scale production [144]. That is a result of them being of small size and with similar density to water, as well as having a negatively charged surface [145]. To confront this challenge, microalgae cultivation could be integrated into a substrate or support structure, facilitating the separation process and lowering the hydraulic retention time. However, the

Table 4
Application of AI and ML tools for Chlorella cultivation, wastewater bioremediation, and bioproduction.

Cultivation and wastewater treatment						
Microalgae species	**Application**	**AI/ML algorithm used**	**Input parameters**	**Output parameters**	**Results**	**Ref.**
Chlorella vulgaris	Nutrients removal from wastewater	ANN	Initial concentrations of PO_4^{3-} and NH_4^+, light intensity, and initial biomass	Ions' concentration in the effluent	The model showed a high correlation between the simulated and experimental results with $R^2 > 0.95$.	[120]
Chlorella vulgaris	Removal of several pollutants from wastewater	Multilayer feedforward ANN	Initial pollutant concentration and contact time	Removal efficiency	A high correlation coefficient value with R^2 values >0.99 and low MSE (<0.1) was achieved.	[121]
–	Microalgae cultivation in nutrient-supplemented treated wastewater	Three-layer feedforward backpropagation ANN	Temperature, pH, TN, TP, dissolved O_2, and electric conductivity	Biomass growth and algal dry cell weight	The developed ANN model achieved high prediction accuracy with $R^2 \sim 0.983$	[122]
Chlorella vulgaris	Microalgae cultivation for maximizing lipid content in biomass	Feedforward ANN	Initial concentrations of glucose, NO_3^-, and PO_4^{3-}	Lipid content and biomass productivity	At the optimized medium conditions suggested by the obtained model, they achieved 1.94 g/L lipid production and 0.31 g/L/day biomass productivity.	[123]
Chlorella pyrenoidosa	Removal of heavy metals from wastewater	ANN	Initial pollutant concentration, pH, inoculum size, and contact time	Removal efficiency	The model showed a superior prediction accuracy for the phycoremediation of ions from wastewater, with $R^2 \sim 0.999$	[124]
Chlorella vulgaris	Phosphorus removal from municipal wastewater	xT SAAM	Initial biomass and phosphorus concentrations and time	Phosphorus removal efficiency	Using the suggested initial conditions led to an increase in the pollutant removal efficiency by 101.7 % and 138.0 % more polyphosphate accumulation.	[125]
Chlorella vulgaris	Cultivation using recycled medium	IoT-based framework	Recycled-to-fresh medium ratio	Biomass yield and content of lipids, protein, and carbohydrates	The achieved model showed result interpretation with high accuracy ($R^2 > 0.92$) based on the trained model performance.	[126]

Biomass conversion and bioproduction						
Microalgae species	**Conversion process**	**AI/ML algorithm used**	**Input parameters**	**Output parameters**	**Results**	**Ref.**
Chlorella pyrenoidosa	Acid catalytic direct transesterification	ANN	Temperature, reaction time, concentration of catalyst, and solid-biomass ratio	Yield of FAME	The model predicted the optimum conditions to obtain the highest yield of FAME with high accuracy ($R^2 \sim 0.94$)	[130]
Chlorella CG12	Supercritical methanol transesterification	RSM, ANN, and GA	Temperature, reaction time, and methanol-to-oil ratio	Yield of FAME	The ANN model, coupled with the GA-obtained optimized conditions, predicted a 99.16 % conversion efficiency ($R^2 \sim 0.99$)	[131]
Chlorella	Hydrothermal liquefaction	GBR and RF	Algal compositions and process conditions	Bio-oil yield and O_2 and N_2 content in bio-oil	The results obtained from GBR exhibited better performance for both single and multi-target task prediction compared to RF with $R^2 \sim 0.90$.	[132]
Chlorella vulgaris	Thermal conversion	PDSE neural network	Temperature, heating rate, catalyst type	Biomass conversion	At the suggested input variables, they obtained a high biomass conversion of 88.3 % from the microalgae.	[133]
Chlorella pyrenoidosa, minutissima, protothecoides, and vulgaris	Pyrolysis	Deep neural network	Temperature and heating rate	Biofuel yield	The obtained model showed a high regression coefficient ($R^2 \sim 0.997$) and a low MSE of 10^{-6} for biofuel production.	[134]
Chlorella vulgaris	Formic acid-mediated hydrothermal carbonization	Bayesian regularization ANN	Temperature, reaction time, pH, feedstock-to-suspension ratio and combined severity factor	Hydrogen formation, hydrochar yield, and liquid phase composition	The developed model showed a high prediction accuracy with $R^2 \sim 0.997$ for the conversion of biomass to green hydrogen	[135]
Chlorella vulgaris	Dark fermentation	NumPy and SciPy package	Thermal pre-treatment duration	Biohydrogen yield	The model achieved high prediction accuracy with $R^2 \sim 0.956$ and an optimum biohydrogen production of 387.1 mL/g.	[136]
Chlorella sorokiniana	Pyrolysis	ANN and RSM	Temperature, heating rate, reaction time, and particle size	Yield of biofuel and value-added chemicals	The model obtained from ANN demonstrated a high prediction accuracy with $R^2 \sim 0.998$	[137]

attached cultivation has some drawbacks, such as fouling and the necessity for mechanical biomass-collecting systems [33]. For biofuel production via thermochemical conversion of microalgal biomass, harvesting the microalgae was evaluated to account for 30 % of the final production cost [146]. Hence, developed harvest methods with low cost and energy requirements are essential for the application of *Chlorella* in large-scale wastewater treatment operations and bioproduction. Furthermore, it is essential to determine whether the biomass obtained after the bioremediation of wastewater can be directly utilized to produce bioproducts, as some contaminants are considered toxic and can pose a risk to the environment.

The economic sustainability of microalgae processes is considered by evaluating techno-economic analysis (TEA) and profitability. However, the TEA of microalgae production is known to be inconstant. The main factors affecting the TEA of this process and contributing to its variability include the design of the cultivation system, medium source and content, scale of biomass productivity, and employed downstream methods [147]. Cultivating microalgae in wastewater presents a promising approach to enhancing the sustainability of zero-waste microalgae-based industries. For instance, cultivating *Chlorella vulgaris* in wastewater to treat emerging contaminants and converting its biomass into biochar as a fertilizer showed great economic potential. The TEA of the integrated phycoremediation and pyrolysis system revealed a net annual profit of $596, accompanied by a payback period of 6.2 years [148]. Integrating bio-oil production from microalgal biomass with protein extraction can enhance revenue by selling extracted proteins, advancing zero-waste microalgal biorefineries [149]. In a similar biorefinery concept, pigments from *Chlorella* sp. were extracted before biodiesel generation, followed by producing sugars and recovery of protein hydrolysate [150]. Furthermore, TEA indicates that co-producing biodiesel and ethanol from microalgal biomass offers economically viable investment opportunities with minimal environmental impact [151].

6.2. Future perspectives

Among the various fields and applications in which *Chlorella* shows potential, its future for large-scale bioremediation of wastewater seems most promising. Being a sustainable approach for the removal of various contaminants with good performance, it became one of the most current hot topics for research. The commercialization and utilization of *Chlorella* on a large scale is dominantly limited to its cultivation in freshwater or seawater [152]. Most current studies were performed on a lab scale, where cultivation conditions and medium and wastewater composition are controlled. Hence, these results may differ from using real wastewater under actual conditions and characteristics. Nevertheless, several studies tested *Chlorella* using real wastewater samples and possessed excellent performance. In addition, the elimination of other emerging pollutants, such as microplastics, using *Chlorella* species is limited, hence further research should be conducted. Another promising implementation of *Chlorella* in wastewater treatment is incorporating the microalgae in nanomaterials and membranes. For instance, Radmehr et al. [153] prepared a hybrid algae-membrane bioreactor for wastewater treatment. They found that the integration of the microalgae caused a decrease in membrane biofouling and higher levels of nitrification compared to the conventional membrane bioreactor.

Genetic engineering of microalgae will play a vital role in future research. It involves the modification, restoration, or enhancement of an organism's genetic material (mainly DNA) to alter its structure or functionality. This emerging technique is promising to improve microalgal processes, mainly in advancing environmental sustainability and biofuel production. Microalgae can be genetically modified to produce cells with enhanced properties that improve their natural abilities for different applications [154]. Introducing innovative microbial genomes into microalgal cells can accelerate the microalgae's development and growth rate [155]. In addition, it can be utilized to enhance the microalgal cell content of bioproducts and their biorefinery processes [156,157]. For instance, genetically modified *Chlorella sorokiniana* exhibited improved biomass productivity (0.71 g/L) and 58.6 % lipid content, demonstrating better properties than the control strain [158]. Up-regulating enzymes controlling starch biosynthesis in *Chlorella vulgaris* led to higher starch content within the produced cells [159]. Thus, genetic engineering shows great potential for overcoming different limitations facing microalgae processing and improving their economic feasibility [160].

Future research into the potential of *Chlorella* for advancing wastewater management and net-zero wastewater discharge and its recovered biomass as future food, fish feed production, and probiotic products are all emerging topics that require further investigation. In addition, advanced methodologies and technologies that facilitate microalgae cultivation at elevated concentrations are still required. These technologies can lead to reduced expenses and more efficient operational durations. The lack of fundamental principles for the design and functioning of microalgal-based wastewater treatment motivates researchers to intensify their efforts and provide essential guidance and endorsements. It enhances the adaptability and robustness of microalgal strains to bioremediate diverse types of wastewaters effectively. Finally, microalgal biomass exhibits significant potential as a source of several bioproducts derived from atmospheric CO_2. However, further research and innovations are needed to compete with fossil fuels and terrestrial food crop feedstocks in terms of cost and quantity.

7. Conclusions

Among the several technologies and methods employed for the elimination of contaminants from wastewater, *Chlorella* microalgae showed its high ability as a sustainable and cost-effective method with good performance. *Chlorella vulgaris* was the most studied species for its application in wastewater bioremediation and bioproduction over the past few years, simultaneously addressing both wastewater treatment challenges and renewable resource demand. Microalgal biomass can generate distinct bioproducts, including biofuels, biofertilizers, biochar, and high-value products, as a sustainable and harmless replacement for toxic and polluting products. AI and ML models can enhance their performance by optimizing different affecting parameters. Furthermore, ML algorithms could be employed to maximize biomass conversion to meet bioproduction goals for different products while reducing costs. The performance of microalgae can be further enhanced by genetically modifying the microalgal cells to improve their properties. The application of *Chlorella* microalgae has a high potential to be commercialized. Nevertheless, further research is still required to encounter the challenges that it still faces for large-scale production.

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CRediT authorship contribution statement

Yazan Abuhasheesh: Writing – review & editing, Writing – original draft, Conceptualization. **Aya Ghazal:** Writing – review & editing, Writing – original draft, Conceptualization. **Doris Ying Ying Tang:** Writing – review & editing. **Fawzi Banat:** Writing – review & editing. **Shadi W. Hasan:** Writing – review & editing. **Pau Loke Show:** Writing – review & editing, Supervision, Funding acquisition.

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.

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Data availability

No data was used for the research described in the article.

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