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Review

Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts

Ashfaq Ahmad [a],*, Fawzi Banat [a],*, Habiba Alsafar [b], Shadi W. Hasan [a]

[a] Department of Chemical Engineering, Khalifa University of Science and Technology, P.O. Box 127788, Abu Dhabi, United Arab Emirates
[b] Department of Biomedical Engineering, College of Engineering, Khalifa University of Science and Technology, P.O. Box 127788, Abu Dhabi, United Arab Emirates

HIGHLIGHTS

- Microalgal application for bioremediation, biofuel, and biomedical is discussed.
- Treatment efficacy, nutrient uptake pathway and challenges are discussed.
- Challenges in algal biofuel production and techno-economic analysis are discussed.
- Integrated microalgal technologies have the potential to generate additional revenue.

GRAPHICAL ABSTRACT
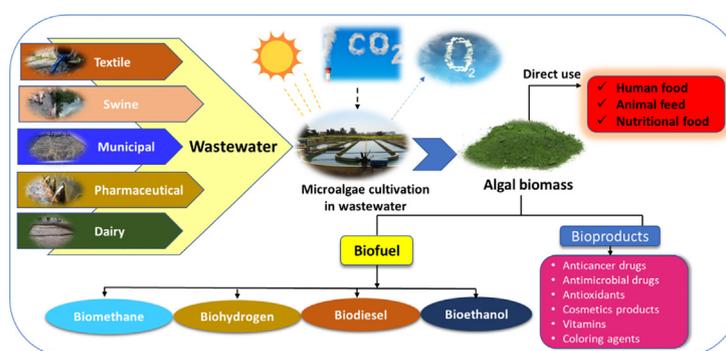
ARTICLE INFO

ABSTRACT

A growing world population is causing hazardous compounds to form at an increasingly rapid rate, calling for ecological action. Wastewater management and treatment is an expensive process that requires appropriate integration technology to make it more feasible and cost-effective. Algae are of great interest as potential feedstocks for various applications, including environmental sustainability, biofuel production, and the manufacture of high-value bioproducts. Bioremediation with microalgae is a potential approach to reduce wastewater pollution. The need for effective nutrient recovery, greenhouse gas reduction, wastewater treatment, and biomass reuse has led to a wide interest in the use of microalgae for wastewater treatment. Furthermore, algae biomass can be used to produce bioenergy and high-value bioproducts. The use of microalgae as medicine (production of bioactive and medicinal compounds), biofuels, biofertilizers, and food additives has been explored by researchers around the world. Technological and economic barriers currently prevent the commercial use of algae, and optimal downstream processes are needed to reduce production costs. Therefore, the simultaneous use of microalgae for wastewater treatment and biofuel production could be an economical approach to address these issues. This article provides an overview of algae and their application in bioremediation, bioenergy production, and bioactive compound production. It also highlights the current problems and opportunities in the algae-based sector, which has recently become quite promising.

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* Corresponding author.
  E-mail addresses: ashfaq.ahmad@ku.ac.ae (A. Ahmad), fawzi.banat@ku.ac.ae (F. Banat).

Contents

1. Introduction

The rapid growth of the global population has exacerbated the environmental challenges of the world. Major types of environmental pollution, such as air, land, and water pollution, contribute to ecological imbalance and global warming. Various toxic substances, including heavy metals, nuclear waste, chemical fertilizers, pesticides, hydrocarbons, and pharmaceutical by-products, contribute to pollution, causing substantial environmental harm (Ahmad et al., 2016). This problem poses a significant challenge to science and requires thorough knowledge and technological solutions. Several physicochemical and biological treatment methods have been developed to remove or remediate contaminants from affected environments. Common physicochemical remediation methods for wastewater treatment include membrane filtration, ion exchange, electrochemical treatment, osmosis, precipitation, and evaporation. However, most of these methods are neither commercially feasible nor environmentally sustainable. Therefore, a new integrated technology is needed to reduce cost and energy consumption (Crini and Lichtfouse, 2019; Nur Hazirah et al., 2014; Rani et al., 2019). Hence, biological wastewater treatment has been preferred over chemical treatments in recent years. Although chemical wastewater treatment is the fastest, the treated wastewater contains synthetic chemicals that have a variety of negative effects on the environment and living organisms. Therefore, biological wastewater treatment is the most environmentally friendly option. Fig. 1 shows the treatment of microalgae wastewater and its applications in various industries.

Biological remediation, or bioremediation, is one of the most promising techniques due to its low cost, ecological benignity, and long-term effectiveness. Bioremediation uses microorganisms to detoxify or reduce the levels of organic or inorganic pollutants in the environment. Different microorganisms have been used in bioremediation, including bacteria, fungi, and microalgae, to remove contaminants from the environment (Abinandan and Shanthakumar, 2015; Blánquez et al., 2008; Shahid et al., 2020). In particular, bioremediation occurs through two main mechanisms: Biosorption and Bioaccumulation. Bioaccumulation is an active bioremediation process in which living organisms absorb the chemicals through their cell wall. Meanwhile, biosorption is a new technique for eliminating heavy metal ions from acidic solutions using living algae or inert biomass. The sorption process is crucial for algal growth. Influenced by environmental variables, adsorption is a complex process in which algal cells take up heavy metal ions during their growth stages (Pavithra et al., 2020). In contrast, passive bioremediation involves physicochemical processes realized by living or dead microbial cells. The exploration of resources that can be used to reduce heavy metal levels in the environment has led to the discovery of unconventional materials that can serve as economical, reliable and safe tools for wastewater treatment. Against this background, biological materials have emerged as environmentally friendly and cost-effective alternatives. Because of their ability and adaptability to resist polluted water, the use of microalgae as biosorbents has been highlighted. Although there has been much research on the bioremediation of polluted environments by various microorganisms, microalgae-based bioremediation technology has received considerable attention as a viable technique due to its advantages. Bioremediation using microalgae can remove contamination and the biomass produced during the process can be converted into other value-added bioproducts (Kalra et al., 2021). The biological method is the most promising for the long-term treatment of industrial wastewater. It could also have the potential to contribute to the development of more circular economic practices.

The concept of using green algae as a feedstock for biofuel production has attracted enormous attention due to rising oil prices, the rapid depletion of natural oil reserves, and most importantly, the disastrous effects of fossil fuel use due to global warming. According to the Organization's World Oil Outlook Report of Petroleum Exporting Countries (OPEC) for 2020, oil is expected to remain the dominant fuel in the future, with global demand rising from nearly 100 million barrels per day (mb/day) in 2019 to approximately 109 mb/day in 2045, although current oil reserves held by members of OPEC are rapidly depleting
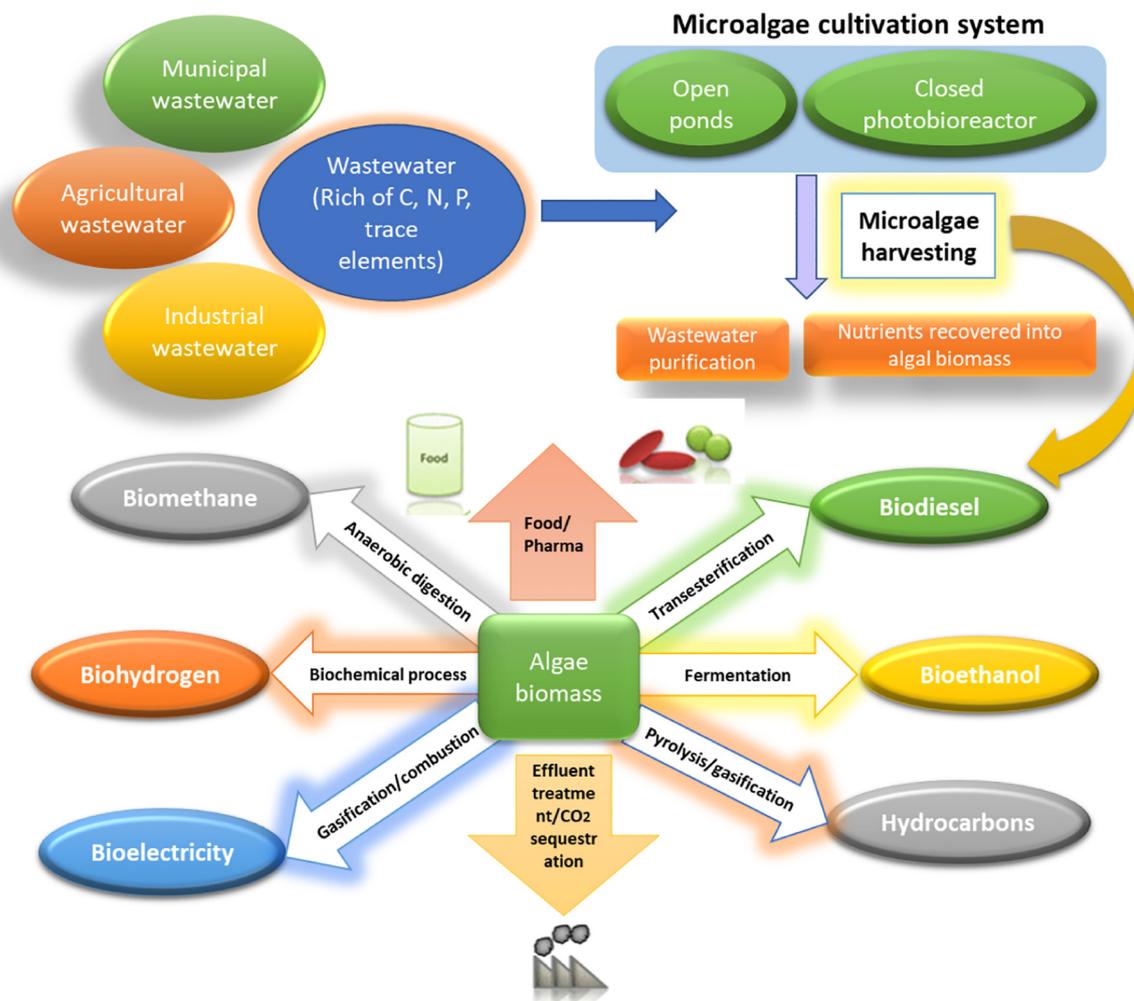
Fig. 1. Adapted: Wastewater remediation using microalgae and its application in various fields.
Reproduced and modified from Aishvarya et al. (2015) and Liu and Hong (2021a) with permission.

(Luna Loya, 2021). Fossil fuel resources will not be accessible in the future. A significant portion of the world's energy needs can be met by renewable energy resources that produce little or no carbon dioxide (CO_2) and provide a large portion of the world's energy needs at a cost similar to current petroleum costs (Johnson and Wen, 2010; Pahunang et al., 2021). Soybeans, sugarcane, and vegetable oils are used to produce first-generation biofuels. Concerns about future food production versus energy demand have shifted the focus to second-generation biofuels. Non-food biomass sources, such as grass straws, wood, jatropha, switchgrass, and organic waste, produce more net energy per acre than maize and sugarcane. However, the complicated thermochemical and biochemical processes required to produce biofuel from these resources consistently result in poor yields and high prices. The ultimate source of third-generation biofuels could be photosynthetic thallophytes. Microalgae have more environmental and economic benefits than other feedstocks and sufficient supply to meet increasing demand while minimizing environmental impacts. Microalgae have already been used to produce biodiesel, bioethanol, and biohydrogen (Yap et al., 2021). Alternative fuels have become very important in many sectors, but especially in transportation. Biofuels from microalgae have attracted public interest as a potential solution to all these problems, both in terms of production and consumption. The benefits of microalgal biomass as a source of biofuel and the production of value-added bioproducts have been widely researched (Jiang et al., 2021; Saravanan et al., 2021).

2. Microalgae wastewater treatment

Commercial industries produce significant pollution that is discharged into the water stream. Depending on the type of industry, most wastewater generated contains a variety of contaminants, including minerals, volatile organic compounds, oils and greases, heavy metals, and pesticides. Additionally, industrial or sewer wastewater is nutrient-rich, and the accumulation of certain pollutants (nitrogen and phosphorus) can significantly affect both freshwater and saltwater ecosystems. Some of these contaminants must be removed before the water can be safely discharged into the environment and made safe for human consumption. Utilizing these nutrients from wastewater is critical for microalgae to perform their cellular functions and produce valuable biomass. Cultivation of microalgae in wastewater can be useful and economically viable, especially given the environmental problems caused by a large amount of wastewater containing harmful and toxic chemicals. In recent decades, intensive research efforts have been made in the field of wastewater treatment and several promising wastewater recycling methods have been investigated (Edokpayi et al., 2017; Kalra et al., 2021; Klimaszyk and Rzymski, 2017). Pollution of water causes various problems, including water scarcity for drinking water and other essential services in households and industry. Therefore, wastewater treatment is necessary to improve water quality and reduce water scarcity (Karimi-Maleh et al., 2020). Recent studies have shown that microalgae bioremediation is a potentially helpful method

for industrial wastewater treatment. The development of microalgae is primarily dependent on the availability of adequate nutrients in the growth medium to ensure the formation of useful products. Micronutrients, vitamins, trace minerals, and macronutrients, nitrogen, and phosphorus are essential for the optimal growth of microalgae (Ahmad et al., 2016). Most of the organic and inorganic nutrients discovered to be beneficial for microalgae culture are present in wastewater. Successful results require treatment with multiple microalgae strains. Microalgae strains such as *Chlorella* sp. (Ahmad et al., 2018; Hariz et al., 2019), *Scenedesmus* sp., *Nannochloropsis* sp. (Emparan et al., 2020b), *Chlamydomonas* sp. (Ding et al., 2016), and *Dunaliella salina* (Choi et al., 2018; Takriff et al., 2016) have been used for wastewater treatment.

2.1. Municipal wastewater treatment

Municipal wastewater is mainly disposed of by households. They are usually discharged untreated into sewers, lakes, and rivers, which hinders the development of communities. Physicochemical properties of municipal wastewater include pH, COD, BOD, total nitrogen, phosphate, potassium, metals, and total microbial load (Ibrahim et al., 2020). Most research studies in this area have reported that municipal wastewater as a medium for cultivating microalgae can increase their biomass production and also eliminate pollutants, including nutrients such as nitrate and phosphate (Ye et al., 2020; Qu et al., 2020). Municipal wastewater was used as a growth medium for cultures of *S. obliquus* and *Desmodesmus* sp. with more than 75% of nitrogen and phosphorus eliminated. The fatty acid composition showed that increasing the light intensity from 50, 150, and 300 $\mu E\ m^{-2}\ s^{-1}$ resulted in a higher content of oleic acid (18:1) and a lower content of linolenic acid (18:3). These results suggest that optimal light intensities are important to improve biomass productivity and fatty acid synthesis of microalgae for high-quality biodiesel production. In addition, a significant reduction in protein content was achieved with a concomitant increase in fatty acid content (Nzayisenga et al., 2020). Table 1 shows the different types of municipal, agricultural, and industrial wastewater used for microalgae growth and removal. The nutrients in wastewater provide food for microalgae and allow them to develop and build biochemical components in their cells. Several studies have reported that culturing microalgae in diluted wastewater results in maximum removal of

nutrients. A similar mechanism was observed with *Chlorella vulgaris* during bioremediation of raw vineyard wastewater. Maximum biomass content (2.63 g_{DW}/L) and biomass productivity of 0.66 $g_{DW}/L\cdot day$ were achieved in 20% (v/v) wastewater after 4 days of treatment. Coculture was able to reduce COD and polyphenol content by more than 92% and 50%, respectively. This study shows that it is possible to use vineyard wastewater as a culture medium for microalgae growth to reduce production costs and to use the resulting biomass as a source of biofuels (Spennati et al., 2020).

2.2. Textile wastewater treatment

Wastewater from the textile industry contains numerous pollutants, especially dyes, that can have negative impacts if not properly treated, such as adverse effects on esthetics, eutrophication, reduced photosynthetic activity, and bioaccumulated toxins in aquatic ecosystems. Microalgal growth in textile dye wastewater has been discovered as a potential alternative to conventional wastewater treatment processes. Dyes in wastewater are degraded during microalgae growth through a bioconversion/biodegradation or biosorption process. Therefore, microalgae treatment could eliminate dye and nutrient contamination of textile wastewater and mitigate various negative environmental impacts caused by its release into the aquatic environment. Furthermore, in bioremediation of textile wastewater, microalgae offer the added benefit of producing valuable biomass that can be converted into bioproducts, biofuels, and bioenergy compared to conventional treatment methods (Premaratne et al., 2021; Sharma et al., 2021). Microalgae and commercial activated carbon were used to remove Reactive Red 120 (RR-120) textile dye from aqueous wastewater. At pH 2 and room temperature, *Spirulina platensis* was removed up to 94-99% compared to 94-98% of the activated carbon dye (Cardoso et al., 2012). In another study, mixed microalgal consortia (Chlorella and Scenedesmus sp.) were used to treat textile wastewater in a fed-batch reactor. The fed-batch reactor was operated for five cycles, each lasting 30-10 days, implying a gradual adaptation of the microalgae. The highest removal of total nitrogen (70%) and total phosphorus (95%) was achieved. The color of textile effluent was removed 68-72%. This study suggests that integrated microbial algal cultivation with textile wastewater could be a viable option (Kumar et al., 2018). Bioremediation of wastewater with microalgae can be performed with both free

Table 1
Microalgal nutrient removal, resource recovery, and lipid accumulation potential in wastewater.

Description	Microalgae	Removal (%)			Biomass	Lipids content	References
		COD	N	P			
Municipal wastewater	*Anabaena* sp.	98.6	100	96.5	215.7 mg/day	7.24	(Hena et al., 2015)
	Chlorella zofingiensis	–	93	90	2.4 g/L	21.6-25.4	(Zhou et al., 2018)
	Scenedesmus obliquus	85.43	80.30	95.72	–	46.92	(Qu et al., 2020)
	Scenedesmus sp. HXY2	96	96.6	94.5	–	15.56	(Ye et al., 2020)
	Scenedesmus obliquus	–	78.5	95.2	0.529 g/L	21.9	(Eida et al., 2018)
	Scenedesmus obliquus	99	99	100	2.68	39	(Pandey et al., 2019b)
Textile wastewater	*Chlorella* sp. WuG23	75	75	–	58 mg/L/day	–	(Wirth et al., 2018)
	Scenedesmus abundans	86.87	68.86	70.79	10.80 mg/L/day	–	(Brar et al., 2019)
	Anabaena ambigua	50	52.95	63.05	11.6 mg/L/day	–	(Brar et al., 2019)
Dairy effluent	*Chlorella vulgaris*	80.62	85.47	65.96	0.175 mg/L/day	–	(Choi, 2016)
	Arthrospira platensis	98.4	98.8	100	0.52 g/L/day	158 mg/L/day	(Hena et al., 2018)
	Scenedesmus sp. ASK22	90.5	100	91.24	1.22 cdw/L	30.7	(Pandey et al., 2019a)
	Acutodesmus dimorphus	90	100	–	–	–	(Chokshi et al., 2016)
Swine wastewater	*Tribonema* sp.	56.6	89.9	72.7	–	42.4	(C.-Y. Chen et al., 2020)
	Tribonema sp.	52.5	100	68-74	2.04 g/L	26.3-55.4	(Huo et al., 2020)
Molasses wastewater	*Monoraphidium* sp. FXY-10	92.33	80	86	1.21 g/L	92.33	(Dong et al., 2019)
	Scenedesmus sp. Z-4	87.2	90.5	88.6	–	28.9	(Ma et al., 2017)
Piggery wastewater	*C. vulgaris*	–	100	100	–	35-40	(Molinuevo-Salces et al., 2016)
Aquaculture wastewater	Microalgal consortium of *Euglena gracilis* and *Selenastrum*	56-68	89	84-96	–	84.9 mg/L	(Tossavainen et al., 2019)
Domestic wastewater	*Scenedesmus* sp.	–	98.8	97.7	0.223 g/L/day	34.3 mg/L/day	(Ren et al., 2019)
	Scenedesmus sp.	69-96	94-98	73-82	0.196 g/L/day	65.2 mg/L/day	(Nayak et al., 2016)

and immobilized algal cells. Bioremediation using immobilized microalgae cells has been shown to have several benefits over freely suspended microalgal cells. The immobilization method has the advantage of requiring less space and making microalgae cells easier to harvest. A study of the immobilized *Desmodesmus* sp. *dye destination* showed that it removed methylene blue and malachite green by up to 98% after 6 days of cultivation (Al-Fawwaz and Abdullah, 2016). Furthermore, Kassim et al. (2018) observed decolorization and nitrogen removal rates of 80% and 71%, respectively, from textile wastewater at a pH of 12, 1000 lux intensity and 150 microalgae beads. Immobilized *Tetraselmis* sp. and *Chlorella* sp. Wu-G23 was also discovered for wastewater bioremediation (Adam et al., 2015; Wu et al., 2021). Wastewater characterization is critical when using wastewater-based microalgae growth as a promising method to reduce biomass production costs, as a high degree of certainty is required to obtain contaminant-free biomass (Jaramillo and Restrepo, 2017). When using wastewater, it is critical to understand the amount of each nutrient, including the N:P:K ratio for microalgae growth. Furthermore, the total organic load (dissolved and suspended solids) of the wastewater is expressed by the COD content. Water discharged from industrial plants has a high concentration of organic matter (e.g. total suspended solids, BOD, COD, minerals, oils, fats, heavy metals, and nutrients such as ammonia salts and phosphate) (Abdel-Raouf et al., 2012; Kalra et al., 2021; Su, 2021).

2.3. Agricultural wastewater treatment

Agricultural wastewater is waste produced in processing commodities such as palm oil, coffee beans, cassava, and sugarcane. Agribusiness is the main consumer of freshwater and livestock is now a major source of wastewater (Ummalyma et al., 2021). The agro-industrial and food processing industries produce enormous amounts of wastewater, as residues and byproducts are unavoidable in any productive sector. When wastewater is discharged into water bodies without being treated to remove nutrients and organic carbon, it leads to eutrophication, which promotes the growth of undesirable species such as aquatic macrophytes and toxin-producing cyanobacteria (Abdel-Raouf et al., 2012). Palm mill oil effluents (POME), instant or lyophilized coffee, cassava (flour and starch), and vinasse from sugar cane biorefinery are all processed in the plant-based sector. Anaerobic digestion is commonly used as the first stage in early wastewater treatment because it produces a large amount of biogas that can be used as a source of bioenergy, reducing operating costs and thus adding value to the process (Ahmad et al., 2016). Production of one ton of crude palm oil requires about 5 to 7.5 tons of water, which generates a large amount of wastewater in the form of POME (Ahmad et al., 2016). The palm oil sector in Malaysia produces a large amount of POME and CO_2 (Ding et al., 2020). POME is treated on-site using pond systems in two sequential acidification, anaerobic, and aerobic digestion processes (Emparan et al., 2020a; Fernando et al., 2021; Pascoal et al., 2021). POME is treated in low-cost pond systems, with the option of using the biogas produced during anaerobic digestion. However, wastewater retains a high nutrient content, even after aerobic digestion and COD, which measures the total organic load (dissolved and suspended solids) of wastewater discharged into rivers. In a study by Fernando et al. (2021), POME is an alternative growth medium for the synthesis of astaxanthin by microalgae because it contains high concentrations of N and P and low amounts of heavy metals. The C:N:P ratio should be adjusted for microalgae culture according to the physicochemical composition of the effluents from aerobically fermented POME (A.A.H. Khalid et al., 2019). The marine microalgae *Nannochloropsis oculata* and *T. suecica* were cultured in POME as an alternative medium for biomass and lipid synthesis. The highest specific growth rates of *N. oculata* and *T. suecica* (0.21 and 0.20 per day, respectively) and the highest lipid content (39% and 27%) were obtained on day 16. Algal culture in POME medium improved the removal of COD (95%), BOD (97%), TOC (75%), TN (91%), and oil and grease (95%) (Shah et al., 2016). Another study

reported the highest removal of COD (95–98%), BOD (90–98%), TOC (80–86%), and TN (80%) with *N. oculata* and *Chlorella* sp. compared to without involving microalgae (Ahmad et al., 2015). Agribusiness is the main consumer of freshwater, and livestock is now a major source of wastewater (Ummalyma et al., 2021).

The wastewater produced from cassava processing, also known as *Manipueira*, has a high COD due to large organic loads but also has significant concentrations of nutrients (de Carvalho et al., 2018) that could be used for the culture of microalgae integrated into the bioremediation process. The use of microalgae in a horizontal fixed-bed anaerobic reactor to treat cassava starch wastewater removed 86.2-85.5% of COD and total solids (Watthier et al., 2019). The wastewater generated from the processing of cassava was used as a substrate for the growth of heterotrophic microalgae to sequester CO_2 and produce bioremediates. The integration of cassava industrial processes with microalgae is an alternative system that uses the former as a nutrient source for the growth of microalgae biomass and the removal of COD. The integration of cassava industry and microalgae production has evolved from a promise to reality, as reported in the following studies: using *C. pyrenoidosa* (Yang et al., 2008), *Phormidium* sp. (Francisco et al., 2015), *Scenedesmus* sp. (Romaidi et al., 2018), *Chlorella sorokiniana* (Melo, 2020), *Spirulina platensis* (Hadiyanto et al., 2019), and using pilot-scale open pond treatment with *Acutodesmus obliquus* (Selvan et al., 2019). These studies demonstrated a significant removal of COD and chemical components such as nitrate, phosphate, sulfate, chloride, calcium, potassium, magnesium, sodium, phosphorus, ammonia, and organic carbon while producing microalgae biomass.

2.4. Dairy wastewater treatment

Wastewater from various processing streams in the dairy industry can be used as a source of nutrients for the cultivation of *Ascochloris* sp. The use of this wastewater ensured the maximum COD removal rate of 95% and the production of algal biomass in a short period of time. In addition to reducing the diversity of bacterial populations in wastewater, maximum biomass production was achieved (Kumar et al., 2019; Zhu et al., 2019). Microalgae removed up to 89.7% COD after 8 days of cultivation in wastewater from dairy farms without sterilization. Complete elimination of total nitrogen from nitrite and ammonia was achieved after 4 and 6 days of culture. Ammonia nitrogen was reported to be the preferred nitrogen source for microalgae because it is digested directly and requires very little energy for assimilation (Ding et al., 2015). During this integration process between microalgae production and the dairy industry, it was reported that for every ton of *C. vulgaris* biomass produced, approximately 102 tons of liquid digestate from the dairy can be treated with simultaneous removal of N and P. Nitrogen can be found in the environment in a variety of forms, including nitrate, nitrite, nitric acid, ammonium, ammonia, molecular nitrogen, nitrous oxide, nitric oxide, and nitrogen dioxide (Barsanti and Gualtieri, 2014). The symbiotic relationship between microalgae and bacteria can provide several competitive advantages over the growth of pure axenic microalgae in the context of biofuel production. The microalgae-bacteria system substantially reduces the major technological limitations associated with the capital cost of maintaining pure microalgae growth. Furthermore, the symbiotic growth of *Tetraselmis indica* and *Pseudomonas aeruginosa* consortia was compared with axenic growth in dairy wastewater (DWW). The highest biomass production of the consortium was 1454.88 mg/L, which was 38.80% higher than the axenic growth of the pure microalgae culture in DWW. The consortium removed 87.49% of COD, 83.76% of total dissolved nitrogen (TDN), and 79.83% of total dissolved phosphorus (TDP). Thus, the symbiotic microalgae-bacteria consortium increased biomass growth and nutrient removal from wastewater. In addition, it may also be useful in biofuel technologies (Talapatra et al., 2021). In addition, microalgae prefer the ammonic form of nitrogen for their growth. In microalgae, ammonic nitrogen is directly used for amino acid synthesis after two reductions by

glutamine synthetase - glutamate synthase as described in Fig. 2 (Crofcheck et al., 2012).

Eukaryotic microalgae assimilate nitrogen from ammonia, nitrate, and nitrite, which enter the cell by active transport across the plasma membrane. However, absorption is only feasible after two reductions mediated by two distinct enzymes, namely nitrate reductase and nitrite reductase (Crofcheck et al., 2012). Cyanobacteria serve as natural fixers of atmospheric nitrogen, and also have the ability to convert atmospheric nitrogen into ammonia, which can be used to synthesize amino acids and proteins (Barsanti and Gualtieri, 2014; Cai et al., 2013). Ammonia toxicity has a wide range of effects on microalgae development under both mixotrophic and autotrophic conditions (Källqvist and Svenson, 2003; Lu et al., 2018). In an autotrophic experimental environment, microalgae adapted well to a medium containing 50 mg/L ammonium (NH_4^+) but grew insignificantly in media containing 100 and 200 mg/L NH_4^+, respectively, with maximum and specific growth rates similar to those of the control group. At 100 mg/L NH_4^+, the biomass did not increase for six days and then declined, while at 200 mg/L NH_4^+ the reduction occurred on the second day. The results suggest greater tolerance under mixotrophic conditions, with little microalgae growth reported at 100 mg/L NH_4^+, but the same observation was reported for 200 mg/L NH_4^+ (Li et al., 2019).

2.5. Swine wastewater treatment

The primary chemical components present in swine wastewater are carbon, phosphorus, and nitrogen. Several studies have suggested that a variety of microalgae species could be used to purify swine wastewater. Microalgae could use phosphorus in the form of H_2PO_4 and $HPO4$ to convert it into an organic molecule such as ATP, which is a significant source of energy, through a process known as phosphorylation (Fig. 3). Passive absorption, which is controlled by environmental variables such as phosphate content, light intensity, and temperature, allows microalgae to absorb phosphate in the form of polyphosphates from the pond system. However, the passive absorption technique needs further investigation to treat phosphorus-rich wastewater. Microalgae are autotrophic and heterotrophic microorganisms that exhibit great photosynthetic efficiency, rapid growth rates, extensive adaptability to extreme environments, and considerable development under intensive cultivation, making them effective organisms for CO_2 capture. In addition to water and other variables, microalgae require vital nutrients to achieve a high rate of biomass production. The cultivation of *Chlorella pyrenoidosa* in swine waste reduces the high organic content and produces a high amount of lipids (Wang et al., 2012). There are significant economic and environmental benefits in integrating swine wastewater-based microalgae with biodigester technology in a circular economy strategy, as microalgae grow and transform wastewater into raw material for added value products (López-Pacheco et al., 2021). Phosphorus partitioning is affected by the pH of the growth medium, as high pH induces precipitation of phosphorus, which means its availability to the algal cells. In one study, the pH effects were investigated in a combined system with *C. vulgaris* and *Bacillus licheniformis*. The combined system removed the highest phosphorus (92%) compared to algae and bacteria alone, which removed 55 and 78% phosphorus, respectively. This study was carried out at a pH of 7. However, in another experiment, the pH decreased from 7 to 3 due to the phosphorus removal ability, indicating that pH is an important factor in the efficiency of phosphorus removal (Liang et al., 2013).

If the biomass of the high algae pond treatment plant is co-digested with primary sludge to produce electricity or heat, the treatment can be considered energy-positive (Passos et al., 2017). Additionally, this method produces valuable by-products such as biofertilizers, which significantly reduce the cost of bioremediation. Integrating microalgae species into wastewater conditions is essential to produce high biomass while efficiently removing pollutants. Microalgae such as *Chlorella* sp. and *Scenedesmus* sp. are two of the most commonly used microalgae
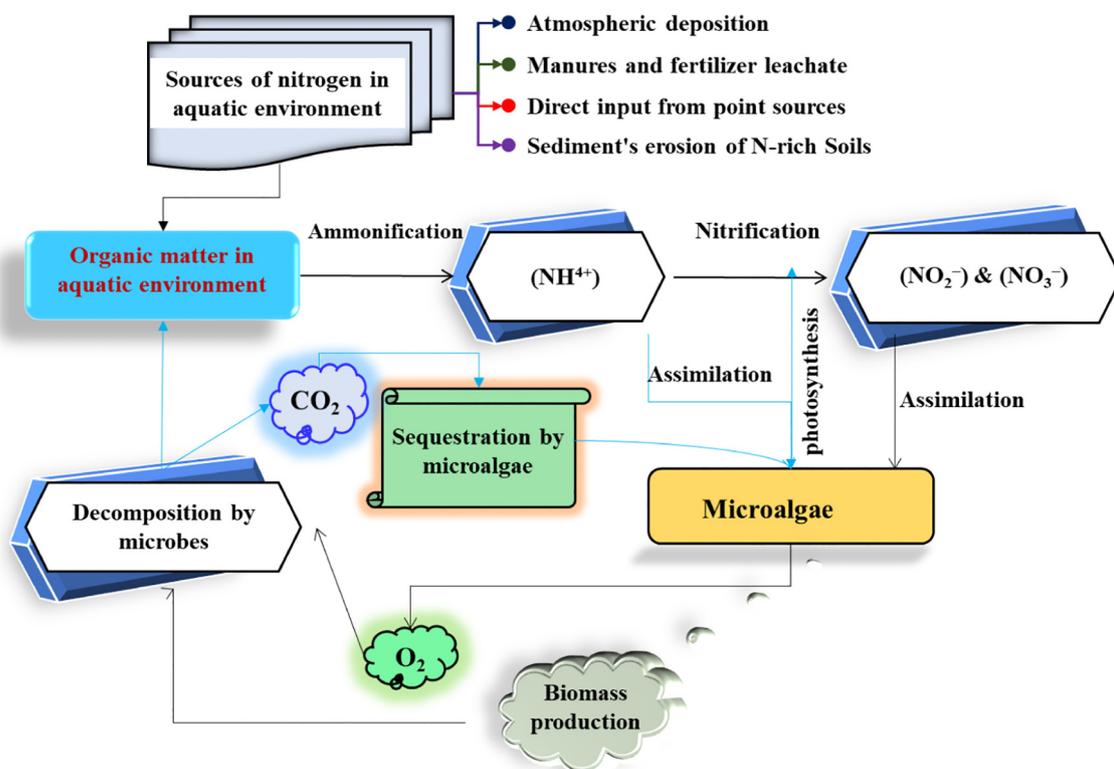

Fig. 2. Adapted: Microalgae's nitrogen removal and carbon sequestration in aquatic environments. Reproduced and modified from Singh et al. (2021) with permission.
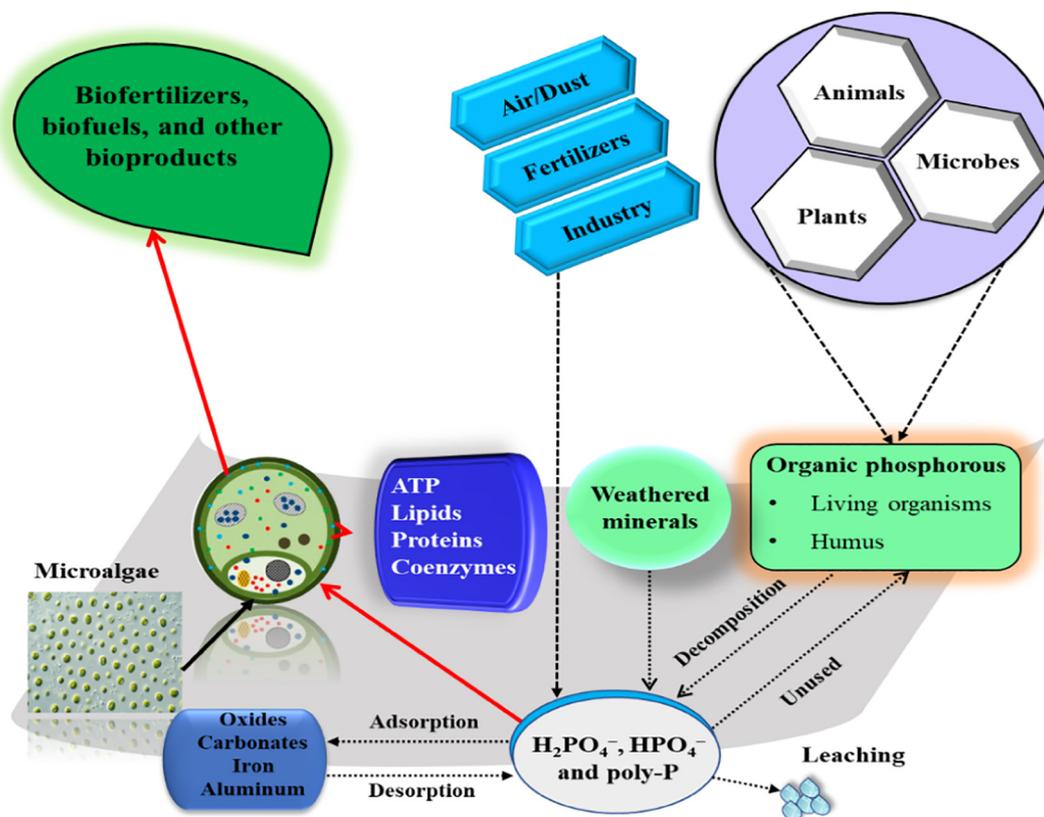
Fig. 3. Adapted: depicts the phosphorus cycle from source to sink, with microalgae originating from various aerial and aquatic sources converting H_2PO_4 and HPO_4 into valuable compounds.
Reproduced and modified from Hussain et al. (2021) with permission.

because of their ability to grow in these conditions. Piggery effluent that is not axenic contains contaminants. Mezzari et al. (2017) suggested the cultivation of *Scenedesmus* spp. as a way to remove *Salmonella enterica serovar Typhimurium* from swine digestate and thus reduce Salmonellosis epidemics caused by animal husbandry. Several researchers have suggested that microalgae are first adapted to wastewater conditions. According to Wang et al. (2017), the most significant removal of COD and NH3-N was achieved with *Neochloris aquatica* in an N/P ratio of 1.5/1. However, before the removal of nutrients from swine wastewater, the balance of nutrients for the growth of microalgae must be determined. Anaerobically digested swine has a large amount of N, P, and micronutrients for the growth of microalgae. Ran et al. (2021) proposed an improved bioremediation method for piggery wastewater using *C. vulgaris* at a mixotrophic growth stage, which increased biomass production to an average of 2.56 g/L. According to Delanka-Pedige et al. (2021), the mixotrophic algal wastewater treatment system ranked first in environmentally friendly wastewater technology, followed by the membrane bioreactor. Research by Cheng et al. (2020b) found that when two auto-flocculation microalgae, *Tribonema* sp. and *Synechocystis* sp., were used, the ammonia and COD removal efficiencies in treated swine wastewater were more significant than those in untreated swine wastewater. The use of *C. sorokiniana* microalgae immobilized on the sponge as a solid carrier in swine wastewater and the reuse of the microalgae-loaded sponge for novel cultivation improved biomass and protein production and the elimination of COD, N, and P (C.-Y. Chen et al., 2020).

2.6. Pharmaceutical wastewater treatment

An antibiotic is a chemical that has antibacterial, antifungal, or antiparasitic properties. Antibiotics are commonly used to prevent and treat infectious diseases in humans and animals. They are also widely used in livestock to promote growth (Kümmerer, 2009). Cephalosporins are a family of β-lactam antibiotics commonly used to treat and prevent bacterial infections by interfering with the production of the peptidoglycan layer of the cell walls of both Gram-positive and Gram-negative bacteria (Magdaleno et al., 2015). Many hazardous chemical compounds, antibiotic residues, and inorganic salts are present in the effluents of cephalosporin producers and pose a threat to the environment and biological life (Guo and Chen, 2015; Yang et al., 2016). In addition, microalgae-based technology has been developed to treat wastewater containing pharmaceutical and personal care products (PPCPs). Several studies have reported that microalgae efficiently remove PPCPs such as antibiotics from wastewater (Villar-Navarro et al., 2018; Xiong et al., 2017). Bioadsorption has been identified as one of the most important processes for the removal of certain antibiotics. It was found that the bioabsorption capacity of microalgae varies depending on the species. Adsorption of 7-amino cephalosporanic acid was achieved with *Chlorella* sp. (4.74 mg/g), *Chlamydomonas* sp. (3.09 mg/g), and *Mychonastes* sp. (2.95 mg/g) in the first 10 min of treatment (Guo et al., 2016). The tetracycline adsorption capacity of the biomass extracted from the lipids of *S. quadricauda* biomass was found to be 295 mg/g (Daneshvar et al., 2018). Moreover, the maximum tetracycline adsorption capacities of *Scenedesmus quadricauda* (295.34 mg/g) and *Tetraselmis suecica* (56.25 mg/g) were obtained under optimized conditions (Daneshvar et al., 2018). *Chlorella pyrenoidosa* showed the highest removal of cefradine (41.47%) after 24 h of treatment with the absorption mechanism, which was 3.4 times higher than the removal achieved without the use of microalgae (12.37%) (Xiao et al., 2021). Bioaccumulation is an active metabolic process in which antibiotics attach to intracellular proteins or other substances in living microalgal cells. Bioaccumulation is influenced by a variety of environmental factors, including temperature,

pH, duration of contact with antibiotics, and concentration of the antibiotic. Biodegradation is the most effective mechanism for antibiotic elimination by microalgae (Xiong et al., 2018). *Chlorella* sp. L38 biodegraded 72% of florfenicol from the medium at an initial concentration of 159 mg/L. This study suggests that *Chlorella* sp. L38 may be a viable alternative alga for the removal of florfenicol from various water sources (Song et al., 2019). In another study, the highest removal of ciprofloxacin (100%) and sulfadiazine (54.53%) was found in Chlamydomonas sp. Tai-03 with carbohydrate production greater than 1000 mg/L/day. According to the elimination methods, the removal of ciprofloxacin was mainly achieved by biodegradation (65.05%), while the removal of sulfadiazine was mainly achieved by photolysis (35.60%) (Xie et al., 2020).

2.7. Heavy metals removal

Heavy metals are one of the most common components of wastewater that produce toxicity in the aquatic environment and harm aquatic animals and plants. Due to their resistance to decomposition, heavy metals enter the food chain and pose a health risk to higher plants and animals, including humans. Some biological materials have the ability to absorb and accumulate heavy metals. Moreover, the use of biomaterials for such purposes is a much more environmentally friendly approach compared to conventional techniques. Previous studies have attempted to identify the most effective biomaterials in terms of heavy metal absorption and accumulation, and microalgae have been identified as the most effective solution in this regard (Hamouda et al., 2016; Zeraatkar et al., 2016). In fact, microalgae have been identified as biosorbents that exhibit greater potential than other types of sorbents. As biosorbents found in aquatic environments, microalgae have attracted significant interest because of their high absorption capabilities, low cost, and natural abundance in most ocean regions around the world. Microalgae possess multifunctional macromolecules that include lipids, proteins, and carbohydrates that have various negatively charged functional groups on their surface, such as amino, hydroxyl, carboxyl, sulfhydryl, sulfate, phosphate, phenol, etc. (Javanbakht et al., 2014). These negatively charged groups allow ions from the surrounding environment to attach, making the outer layer of the cell wall the initial participant in the removal of HMs (Leong and Chang, 2020; Singh et al., 2020). Therefore, it is important to understand the structure, composition, and characteristics of the cell wall for the biosorption of heavy metals (Podder and Majumder, 2017). Furthermore, this non-metabolic process is highly dependent on operating conditions, the impact of physicochemical parameters such as pH, temperature, the presence of other metal ions, and the ratio of adsorbate to adsorbent must be regulated (Zeraatkar et al., 2016). Fig. 4 described the general mechanism of heavy metal elimination. Heavy metals resistant to heavy metals, such as *Nitzschiapalea* and *Nitzschiaperminuta*, can accumulate a significant amount of metals in their bodies, which means they can be used to remediate wastewater containing high levels of heavy metals (Chen et al., 2013). Several methods have been proposed to enhance heavy metal adsorption by microalgae. For example, *Ceramium rubrum* was investigated for its ability to absorb copper from aqueous solutions and was found to have a bioabsorption capacity of 25.51 mg/g of total biomass. Furthermore, its efficiency was found to increase to 42.92 mg/g and 30.03 mg/g when treated with NaOH and CH_2OH, respectively (Imani et al., 2011). In addition, *Phacus* sp. was found to have a higher resistance to thallium in polluted waters. The strain tolerates thallium well due to the pellicle-like coating surrounding its body (Płachno et al., 2015).

Acid-tolerant microalgae *Desmodesmus* sp. MAS1 and *Heterochlorella* sp. MAS3 are capable of absorbing heavy metals such as Fe and Mn. The highest removal of Fe (40–80%) and Mn (40–60%)% was achieved at an
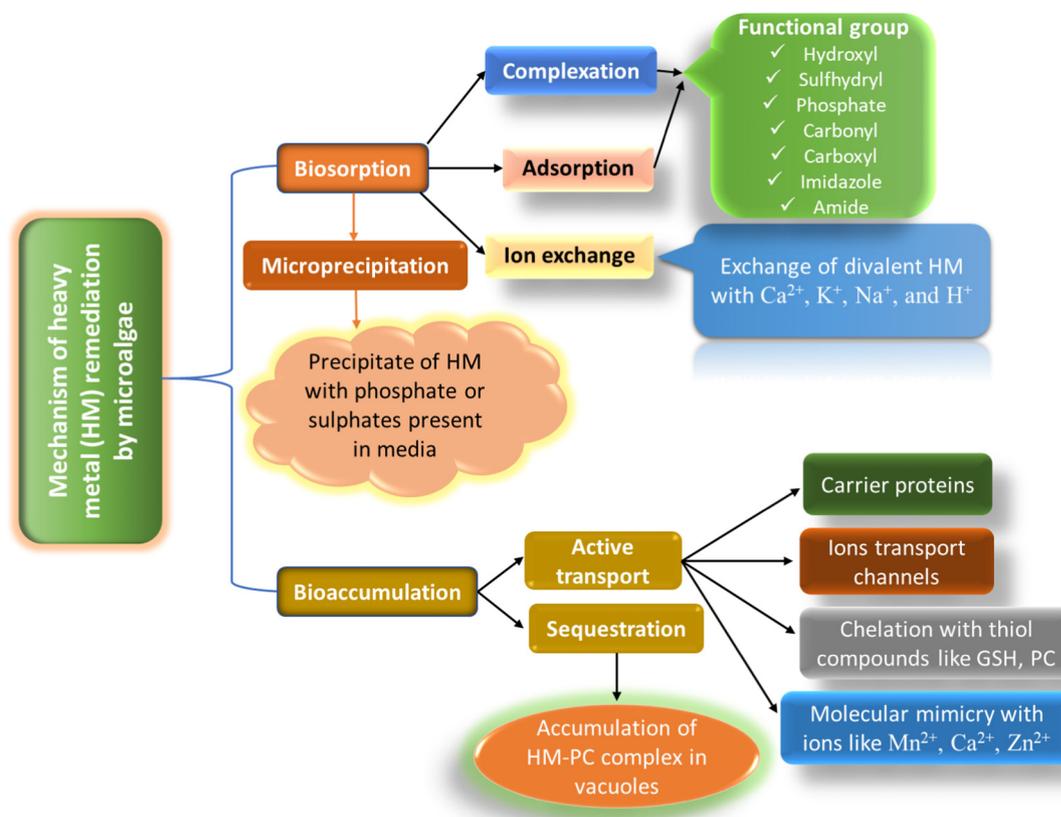

Fig. 4. Adapted: Flow diagram illustrating the mechanism through which microalgae remove heavy metals. Reproduced and modified from Tripathi and Poluri (2021) with permission.

acidic pH of 3.5. Cellular studies suggest that intracellular mechanisms are predominant for the removal of heavy metals in both strains (Abinandan et al., 2019). According to Jiang et al., *Chlorella vulgaris* exhibits strong resistance to arsenic (As(V)) up to 200 mg/L, achieving a constant arsenic removal of 70%. At concentrations, less than 100 mg/L, extracellular adsorption of heavy metals plays an important role in the removal of As(V) by microalgae (Jiang et al., 2011). *Scenedesmus almeriensis* showed the highest removal of As up to 41.7% at optimal pH 9.5 compared to other green microalgae such as *C. vulgaris*, *Chlamydomonas reinhardtii*, *S. almeriensis* and indigenous *Chlorophyceae* spp. (Saavedra et al., 2018). *Chlorella minutissima* UTEX2341 showed high cadmium adaptation, with the highest removal capacity of 35.65 mg/g. Compared to the control, absorption of 0.6 mM Cd (II) improved microalgal lipid content and productivity by up to 42% and 2.17 times, respectively. The highest lipid productivity of 249.36 mg/L·day was much higher than several previously reported values (Yang et al., 2015). In addition to the significant contributions of the phosphate and carboxyl groups, functional groups such as the amide, C—N, hydroxyl, and S—O groups also contribute to the adsorption of Cd (II) in *S. obliquus* AS-6-1. This indicates that cadmium chloride does not affect the flocculation ability of *S. obliquus* AS -6-1 and may contribute to chemical flocculation under certain conditions (Zhang et al., 2016). Heavy metals are a significant component of all wastewaters. Therefore, a comprehensive study on the uptake and accumulation of such metals is needed, including the use of different strains of microalgae under different stress conditions.

2.8. Challenges concerning microalgal wastewater treatment

Industrial activity can generate significant amounts of wastewater that are subsequently released into the environment. In general, wastewater treatment regulations are stringent in countries around the world, and industries are required to treat polluted wastewater before discharge it into the environment to prevent the pollution of streams and rivers. The most commonly used methods for decontaminating industrial wastewater include adsorption, evaporation, chemical precipitation, and filtration, reverse osmosis, oxidation and biological reduction, oxidation and chemical reduction, electrochemical treatment, and ion exchange. Among these methods, ion exchange and adsorption using activated carbon are the most widely used. Both methods are effective but costly, mainly due to the high cost of activated carbon (Crini et al., 2019). The use of chemical oxidation to achieve complete degradation is generally a costly process because the oxidation intermediates generated during treatment become more resistant to complete chemical degradation (Oller et al., 2011). An essential step in the decontamination of industrial wastewater is to expand the scope of available materials for this purpose. The use of algae can reduce the cost of treating industrial wastewater and make the process easier and more widely applicable. Consequently, the growth of microalgae in wastewater generated by industrial processes is not only a sustainable alternative to bioremediation and wastewater treatment, but also a valuable way to generate revenue as microalgal biomass is produced in these processes. Any wastewater treatment method that uses the capacity of microalgae to remove contaminants has the added benefit of being environmentally friendly, which gives it commercial viability (Rahman et al., 2020). Unlike conventional wastewater treatment methods, which are usually costly and unsustainable, microalgae cultivation is a cost-effective alternative that serves two functions: phytoremediation and the production of usable biomass suitable for various applications (Gupta et al., 2016). Importantly, the use of microalgae to treat municipal and agricultural wastewater is environmentally friendly and cost-effective (Gupta et al., 2015; Kumar et al., 2015; Liu and Hong, 2021b).

The use of microalgae is the most efficient method of industrial wastewater treatment; microalgae remove nitrogen, phosphate, heavy metals, and hazardous chemicals, fix CO_2 in the environment (Gupta

et al., 2015; Liu and Hong, 2021a), and detoxify organic and inorganic pollutants in wastewater (Sutherland and Ralph, 2019), which benefits the environment. Capital and operating costs of microalgae wastewater treatment are also lower than conventional treatment methods (Craggs et al., 2012). Additionally, the cultivation of microalgae and potential biomass production can reduce the energy consumption of the system by producing energy and biofuels simultaneously (Das et al., 2021). The most expensive aspect of the process concerns the harvesting of microalgae biomass after wastewater bioremediation (Gupta et al., 2016). This microalgae biomass needs to be harvested regularly to maintain an adequate level of wastewater treatment, and if done incorrectly, it can be expensive compared to the previously mentioned wastewater treatment methods. In fact, harvesting is one of the most expensive expenses in the microalgae treatment system, second only to the cost of installation. A significant amount of investment and running expenditures are required to produce biomass on a large scale (e.g., for use in photobioreactors) (Gupta et al., 2015). Fungal-assisted bioflocculation of microalgae offers a new approach to biomass recovery from wastewater treatment. Co-culture of microalgae and fungi not only reduces harvesting costs but also makes wastewater treatment more cost-effective and provides greater pollutant removal efficiency than monoculture. The stability of an integrated approach of fungi and microalgae is more robust in harsh environments. Fungal-microalgal biomass is ideal for biofuel production from wastewater. However, further research should be conducted on co-cultivation of fungal-microalgal consortia for bioflocculation and wastewater treatment (Leng et al., 2021). Biocontrol fungi and selected microalgae species with excellent adaptability and no contamination risk to the environment need to be evaluated and analyzed for this system. To improve the potential and economic benefits of the system on a large scale, co-culture conditions need to be optimized, such as natural light and pH, fungus/algae ratio, agitation, temperature, and without adding a carbon source (Chu et al., 2021; Leng et al., 2021). It was observed that *C. pyrenoidosa* rapidly flocculated with *Aspergillus fumigatus* pellets. Almost 99% of microalgal cells were flocculated in a short time (3 h). In the same study, algae grown in wastewater reached 95% flocculation efficiency after 3.5 h. This study contributed significantly to the understanding of the processes behind the use of pelleted fungal algae for rapid flocculation (Bhattacharya et al., 2017). In another study, *Chlorella* sp. cells were harvested with an edible fungal strain (*Pleurotus ostreatus*). A maximum harvest efficiency of 65% was achieved within 150 min. This technique is flocculant-free and economical, and the raw material obtained can be used for feed and food production (Luo et al., 2019).

The cost of microalgae biomass production is estimated between $20 and $200 per kilogram, depending on the method used (Brennan and Owende, 2010). However, the efficiency of the wastewater treatment system and its improved performance compared to other techniques make microalgae treatment an economically viable approach (Torres et al., 2014). Taking into account the global environmental crisis, the production of microalgae biomass during wastewater treatment could facilitate energy sustainability, the development of high-value products, recycling, and pollution control (Vieira de Mendonça et al., 2021). The biomass of microalgae also serves to restore the environment and natural resources due to the fixation of CO_2 and the ability to survive a wide range of climatic conditions (Sharma et al., 2019). Ultimately, the microalgae wastewater treatment system is a low-cost and cost-effective option for effluent treatment that is associated with both environmental benefits, such as wastewater treatment and zero carbon emissions and economic benefits, such as biomass production byproducts and energy generation.

3. Biofuel production from algal biomass

Bioenergy is renewable energy derived from natural or biological sources. Bioenergy is often referred to as a renewable and sustainable

energy source. It has recently become an important area of research for scientists around the world. Bioenergy is a great short- and medium-term solution to curb global warming and provide clean energy (Hariz and Takriff, 2017). The demand for biofuels to replace fossil fuels is high because the amount of fossil fuels currently consumed exceeds the amount of fuel produced. For example, according to base case research assumptions, the United States by 2030 would produce 991 million dry tons of biomass per year in low-yield estimates and 1147 million dry tons of biomass per year in high-yield estimates, which is much more than other nations would produce (Khoie and Yee, 2015). Statistically, this amount of biomass can be utilized to produce biofuel, which would only meet 25% of the country's annual energy needs (Khoie and Yee, 2015). During the last decade, the potential of algae as a biofuel feedstock has received much attention. Algal biofuels successfully reduce CO_2 emissions, thus protecting the environment by maintaining a balance between CO_2 production and consumption compared to fossil fuels. Biodiesel combustion releases CO_2 that is absorbed by microalgae. The combustion of biofuels has been shown to emit less CO_2 than fossil fuels (Merlo et al., 2021; Mondal et al., 2017). Biomass-derived biofuels have several advantages, including their regenerative capacity and low impact on pollution and global warming (Khan et al., 2018). Biodiesel from microalgae also helps prevent global warming by reducing CO_2 emissions. Algal biodiesel can reduce CO2 emissions by 78.5% compared to petroleum-based diesel (Van Gerpen, 2005). Algal biofuels offer many advantages over fossil fuels, such as (a) they are readily available from common algal biomass sources, (b) they contribute to the carbon dioxide cycle when burned, (c) they are extremely environmentally friendly, (d) they benefit the environment, the economy, and consumers, (e) they are biodegradable and contribute to long-term sustainability (Ahmad et al., 2016). They are classified into four generations depending on the feedstocks used, the feasibility of production, and the level of technological development (Alalwan et al., 2019). First-generation biofuels produced from conventional crops have a limited supply of feedstock because they are also the primary source of food for humans (Rodionova et al., 2017). Second-generation biofuels produced from agricultural waste or by-products continue to compete with land food production (Chowdhury and Loganathan, 2019). Biofuels from microalgae are categorized as third-generation biofuels because they contribute significantly to first- and second-generation biofuels, which are derived from edible and non-edible resources, respectively. Fig. 5 shows the different processes involved in the conversion of algal biomass to different biofuels.

The main biofuels derived from algae are biodiesel, bioethanol, biogas, and biohydrogen (Hussain et al., 2021). Genetically engineered microorganisms, such as microalgae, yeast, and fungi, are used to produce

fourth-generation biofuels. Various efforts have been made to increase the potential production of microalgae-based biofuels (Chowdhury and Loganathan, 2019; Schenk et al., 2008). Biodiesel is produced by chemical conversion or transesterification of algal oil, while electricity can be generated by burning algal biomass. Similarly, biochemical conversion technology can be used to produce methanol and ethanol from algal biomass through anaerobic digestion and fermentation, which can be used in conjunction with other processes. Thermochemical conversion of algal biomass can be used to produce bio-oil and charcoal (pyrolysis), syngas or fuel gas (gasification), and bio-oil (liquefaction process) (Chiaramonti et al., 2007; Medipally et al., 2015). Biofuels, on the other hand, can pose certain issues about policy, technology, and feedstocks (Fig. 6).

3.1. Biodiesel production

Biodiesel is a sustainable fuel produced from renewable biomass and unused lipids that can replace petroleum diesel (Magda et al., 2021; Milano et al., 2016). The lipid content of algal biomass affects the quality and production of biodiesel and its suitability as an alternative fuel to petroleum-based diesel (Milano et al., 2016). Furthermore, like petroleum-based diesel, biodiesel produced from algal biomass is sulfur-free and has lower particulate matter and greenhouse gas emissions (Schuurmans et al., 2015). However, the oxidation stability of algal biodiesel is limited due to its poor performance at cold temperatures (Chye et al., 2018). Milano et al. investigated the production of biodiesel from microalgae strains with high concentrations of oleic acid in their fatty acids. Biodiesel is produced from microalgae such as *Chlorella* sp., *N. oculata*, *Botryococcus* sp., *Scenedesmus* sp., and *Picochlorum* sp. *Saccharomyces cerevisiae* contains more oleic acid, which helps to improve the oxidative stability of biodiesel (Milano et al., 2016).

Biodiesel is mainly obtained from microalgae via a transesterification process. Transesterification of lipids, especially triacylglycerides (TAG), in the presence of alkali or acid leads to the production of biodiesel and glycerol (Sirajunnisa and Surendhiran, 2016). The selection of microalgae strain is an important factor for biodiesel production (Chye et al., 2018). The cetane number (CN), viscosity, calorific value, and melting point determine the quality of biodiesel and its performance in engines. Bioengineering of microalgae strains is one of the proposed methods to improve biodiesel quality (Nigam and Singh, 2011). The crude oil extracted from algae has a higher viscosity than diesel oil, making it unsuitable for direct use in engines. To lower the viscosity and increase the fluidity of algal oil, a chemical process known as transesterification is required. Esterification is any interaction between a fatty acid (or organic acid) and alcohol that results in the formation
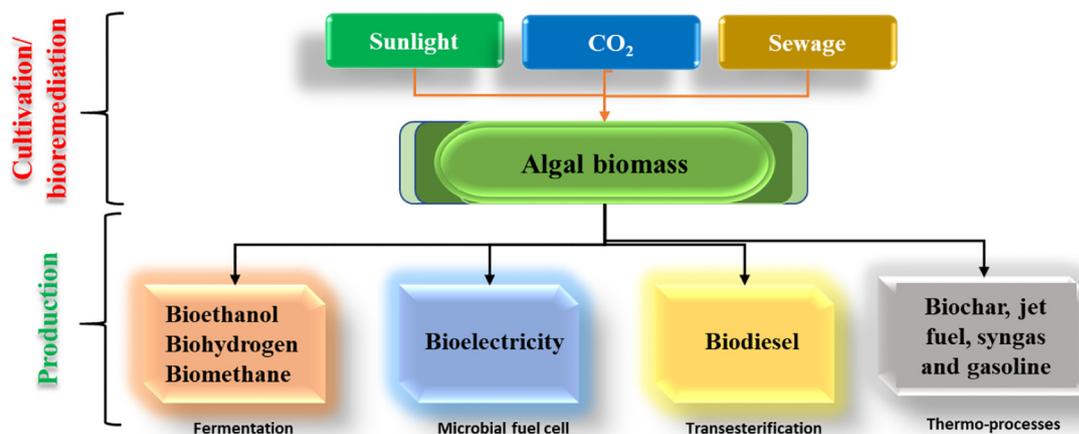

Fig. 5. Adapted: Production of various biofuels from algae biomass. Reproduced and modified from Saad et al. (2019) with permission.
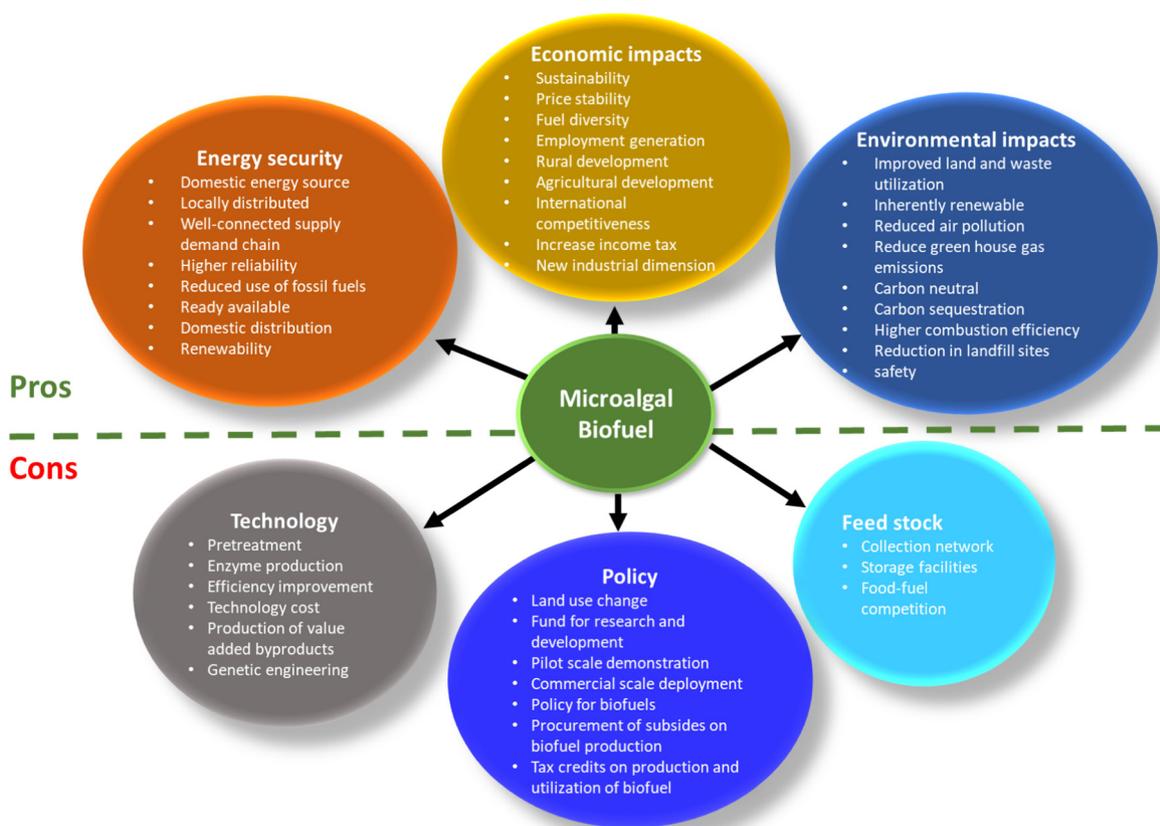
Fig. 6. Adapted: Pros and cons of algal biofuels.
Reproduced and modified from Zabed et al. (2019) with permission.

of an ester. In contrast, transesterification is a reaction between ester and alcohol that results in the formation of fatty acid-alcohol esters by replacing the alkoxy group. Triglycerides are broken down into diglycerides, then monoglycerides, and finally glycerol. Transesterification of large and branched triglyceride molecules results in short and straight-chain esters (Sinha et al., 2008). An experimental study on the production of biodiesel from *Chlorella* species revealed that they have very high fatty acid content and produce 34.53–230.38 mg/L/day of biodiesel in Malaysia (Vello et al., 2014).

3.2. Bioethanol production

Bioethanol is the most successful biofuel currently available that can be produced from microalgae biomass (third-generation). Bioethanol production from microalgae biomass has several advantages, including that it does not require huge areas of arable land and relieves the environment by absorbing CO_2 from the atmosphere. The cell wall of microalgae contains an abundance of lipids and carbohydrates, such as cellulose, mannans, sulfated glycans, xylans, and starch. These complex components are chemically or enzymatically reduced to simple sugars, which are subsequently converted to bioethanol under anaerobic conditions (Chaudhary et al., 2014). Bioethanol is produced from microalgae through several processes, including selection and cultivation of algal biomass, pretreatment, liquefaction, saccharification, anaerobic fermentation, and distillation for bioethanol purification (Vergara-Fernández et al., 2008). Fig. 7 shows a general overview of the synthesis of bioethanol from microalgae with different pretreatment and fermentation methods. Pure ethanol is a gasoline alternative that has both a higher octane number and heat of vaporization compared to gasoline, making it an efficient fuel. It has the same energy content as 66% gasoline by volume (Singh et al.,

2011). Microalgae are considered more effective feedstocks for bioethanol production than traditional crops, such as maize, soya beans, or sugarcane (Dębowski et al., 2013; Zhang et al., 2014).

Bioethanol produced from microalgae has a potential yield almost twice that of sugarcane and five times that of maize (Vergara-Fernández et al., 2008). Several algal species have been identified as significant for bioethanol production, including *Chlorococcum* sp., *Spirogyra* sp., *C. sorokiniana*, *Gelidiumamansii*, *Sargassum* sp., *Gracilaria* sp., *Laminaria* sp., and *Prymnesium parvum* (Behera et al., 2015; Constantino et al., 2021; Rajkumar et al., 2014). *C. sorokiniana* was described as the most successful hydrolysate for bioethanol production with a bioethanol yield of 0.464 g/g reducing sugar and productivity of 0.344 g/L·h (Constantino et al., 2021). The potential of *Chlorococcum humicola* biomass as bioethanol feedstock was also reported. The microalgae were acid hydrolyzed at 120–160 °C (acid concentrations ranging from 0.36 to 3.6 N), resulting in a final bioethanol content of 7.2 g/L from *S. cerevisiae*. Alkali hydrolysis of the same microalgae with NaOH (0.2–0.5 N) at 60–120 °C produced 26% bioethanol (based on $g_{ethanol}/g_{biomass}$) (Harun and Danquah, 2011; Harun et al., 2011b). In another study, enzyme-catalyzed hydrolysis was reported to give high glucose conversion yields (89.8%) for marine algae *P. cruemtum* and 85% for freshwater *P. cruemtum*. For freshwater *P. cruemtum*, simultaneous saccharification and fermentation (SSF) resulted in a higher bioethanol yield of 70.3%, compared to 65.4% for separate hydrolysis and fermentation (SHF). These results indicate that *P. cruemtum* can grow in freshwater and could be a potential bioethanol contender (Kim et al., 2017).

3.3. Biomethane production

Microalgae can also be used to produce biogas, which can then be used to generate electricity, fuel cells, and liquid fuel. Algal biomass
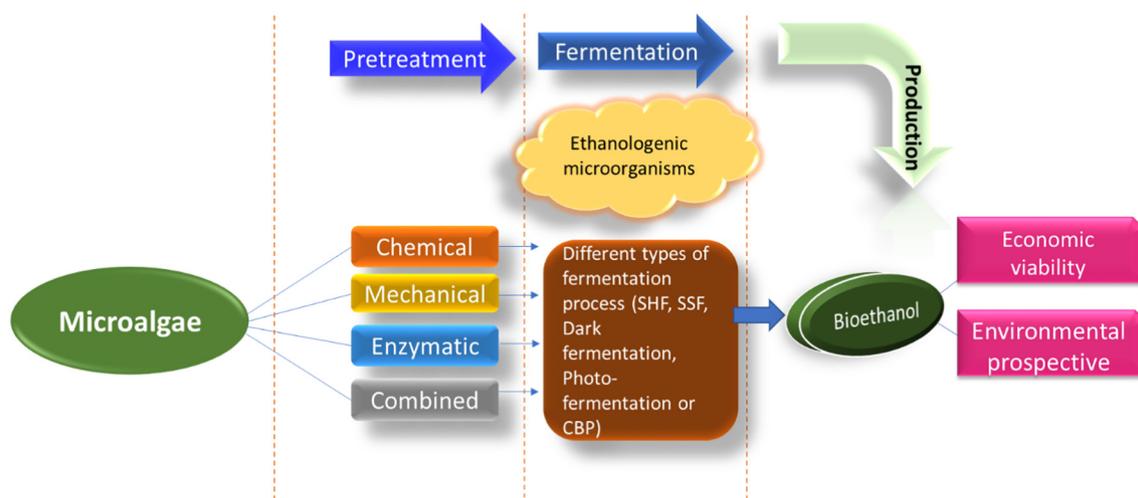
Fig. 7. Adapted: A general overview of bioethanol synthesis from microalgae using various pretreatments and fermentation methods. Reproduced and modified from Phwan et al. (2018) with permission.

contains small amounts of lignin and cellulose, making it an excellent feedstock for the production of biogas by anaerobic fermentation (Harun et al., 2011a). Biogas is the end product of anaerobic digestion, which contains 55-75% methane (CH_4) and 25-45% CO_2 (Chye et al., 2018; Li et al., 2008). Microalgae are an effective source of biogas because they contain higher concentrations of polysaccharides and lipids, no lignin, and lower amounts of cellulose. They are also easier to harvest, require less land for cultivation, are easily converted to biogas, and grow faster than lignocellulosic biomass. Furthermore, anaerobic digestion produces solid waste that is used as a soil supplement (Itskos et al., 2016; Saqib et al., 2013; Zhong et al., 2012). Seaweed has great potential for biogas generation. Many algae species, including *Scenedesmus*, *Euglena*, *Spirulina*, and *Ulva*, have been used for biogas production (Nagarajan et al., 2017; Tiwari and Pandey, 2012). Biogas production involves three steps: hydrolysis, acetogenesis, and methanogenesis (Behera et al., 2015; Brennan and Owende, 2010; Saqib et al., 2013). However, several constraints make biogas production undesirable. For example, the risk of eutrophication and the generation of hazardous chemicals complicate the process (Li et al., 2008; Zhong et al., 2012). The low C:N ratio produces ammonia, which is harmful to anaerobic species, such as bacteria. Sodium ions also suppress microbial activity. Consequently, salt-tolerant microorganisms are recommended for the production of biogas by anaerobic digestion of microalgae biomass (Behera et al., 2015; Brennan and Owende, 2010). Microalgal cultures grown under different conditions showed nitrogen production that increased the C/N ratio to 24-26. Compared to other species, *C. reinhardtii* increased biogas production by 65% (69,823 mL/g VS). Under such conditions, *P. kessleri* and *S. obliquus* generated 70,639 mL/g of VS and 58,636 mL/g of VS biogas, respectively (Klassen et al., 2015). In another study, *A. platensis* was used to produce biogas in the presence of carbon-rich cosubstrates in different C: N ratios, resulting in 4 g VS/L/day biogas (Herrmann et al., 2016). Anaerobic co-digestion of freshwater microalgae *Chlorella* sp. with empty fruit bunches of oil palm (OPEFB) was used for the treatment of POME and biomethane production. The maximum specific biogas production rate was reported to be 0.128–0.129 m³/kg COD/day (Ahmad et al., 2014b). Another study revealed that co-cultivation of marine algae *N. oculata* and POME gave a maximum specific biogas production rate of 1.13–1.14 m³ kg/COD/day. The absence of *N. oculata* and OPEFB co-cultivation resulted in 1.3-fold lower biomethane yield, while the specific biogas production rate remained constant at 1.13–1.16 m3 kg/COD/day (Ahmad et al., 2015; Ahmad et al., 2014a).

3.4. Biohydrogen production

Biohydrogen has attracted much attention as a potential energy source due to its high calorific value and clean oxidation properties. The production of biohydrogen through biological processes is more environmentally friendly, requires less energy, and can be done under ambient conditions than thermochemical processes. Recently, the production of biohydrogen from microalgae has emerged as a potential approach for green energy production (Singh and Das, 2020). Biohydrogen production in microalgae is classified into two types: light-dependent and light-independent (Batyrova and Hallenbeck, 2017). In the first category, water is bio-photolyzed by microalgae and cyanobacteria, while photo-fermentation is mostly carried out by photosynthetic bacteria. In the second category, the process of dark fermentation takes place, in which organic molecules are fermented by anaerobic bacteria (Aslam et al., 2018; Budiman and Wu, 2018). Under anaerobic conditions, microalgae can produce biohydrogen when exposed to light and water. Biohydrogen is considered an efficient energy source because it does not emit greenhouse gases and produces water as a byproduct (Hallenbeck et al., 2016). Compared to other fuels, biohydrogen has the highest gravimetric energy density and conversion efficiency with values of 142 MJ/kg, making it the most efficient fuel (Cuellar-Bermudez et al., 2015). Hydrogenase is a proton-lowering enzyme originally discovered in microalgae that splits water to produce hydrogen under dark anaerobic fermentation conditions (Cuellar-Bermudez et al., 2015). However, a disadvantage of this technique is the limited reaction time due to oxygen generation, which rapidly deactivates the hydrogenase enzyme. Improved techniques are also required to increase the oxygen tolerance of the hydrogenase enzyme, which requires further advances in technology (Chye et al., 2018; Cuellar-Bermudez et al., 2015). Chen et al. (2016) used FSPE biomass of *C. vulgaris* (carbohydrates) pretreated with 1% H_2SO_4. Dark fermentation of the biomass of *C. vulgaris* FSPE with *C. butyricum* CGS5 produced 2.87 mmol H_2/g effectively generated biohydrogen from *Chlorella* sp. (~11.6 mL/gVS) through dark fermentation (Lunprom et al., 2019). Dark fermentation is a low-cost, environmentally friendly technique that also produces by-products such as lactic, butyric, and acetic acid, which can be easily commercialized. This process does not require light source and aeration, which can reduce the additional cost. Therefore, the integrated algae-bacteria system provides an improved sustainable technique to increase the efficiency of biohydrogen production through dark fermentation while reducing the overall cost.

3.5. Challenges in algal biofuel production

Microalgae are a potentially renewable resource that can be used for a variety of applications, including biofuel production, high-value bioproducts, and environmental remediation, such as CO_2 reduction and wastewater treatment. The challenges now facing the industry include algae culture, carbohydrate-rich biomass production, harvesting, biomass pretreatment, and the development of an economical algae system. Algal biomass offers many advantages over first- and second-generation biofuels for biofuel production. Several innovations have been made to advance the biofuels production process from algae. However, biomass harvesting is not yet fully optimized for large-scale production and would benefit from techno-economic improvements for efficiency (Halder and Azad, 2019). Currently, the high cost of algal growth is a major hurdle for the production of algal biofuel compared to the biofuel from other feedstocks. In an open flow-through pond system, contamination of algal cultures affects its biomass production (Dutta et al., 2016). In addition, techniques for harvesting and dewatering the algal biomass are expensive and energy-intensive. Since algal cultures grow in aqueous suspension, harvesting and separation are major challenges in biofuel production (Mutanda et al., 2020). The harvesting process accounts for 20 to 30% of the final production cost (Raheem et al., 2015). Furthermore, harvesting methods are not practical and inefficient in terms of cost and production, as they have not yet been developed. As a consequence, low-cost, highly efficient methods need to be developed. Algal biomass production facilities should be located near a wastewater source, such as industrial or municipal wastewater because algae can grow in wastewater that is high in salt and nutrients. Algae use wastewater nutrients to reduce the need for primary or secondary treatment (Culaba et al., 2020).

The biomass composition of various algal strains varies mainly in terms of carbohydrate, protein, and lipid content. A diverse biomass composition is required for various types of biofuels production. Biodiesel made from microalgae, for example, requires a high lipid content. Culture conditions influence the amount of algal biomass produced. Typical microalgae biomass has high lipid and carbohydrate content while cultivated in a low-nitrogen environment, making it an ideal candidate for biofuel production. The low productivity of biomass and lipid content is an obstacle to the production of a significant amount of algae biodiesel. Therefore, intensive research is needed to find algal strains with fast growth rates and high biofuel yields. In addition, a comprehensive study of optimizing growth factors to increase productivity is important so that it can be implemented on a large scale to achieve higher production in a short time. The stress management strategy should also aim to increase the growth of algal biomass (Halder and Azad, 2019; Mutanda et al., 2020).

The cell wall of microalgae is structurally rigid, which requires pretreatment prior to bioethanol production. Similarly, lipid segregation is required for biodiesel production. These pretreatment processes are expensive, time-consuming, and energy-intensive. Consequently, extensive research into cost-effective pre-treatment technologies that increase productivity and consume less energy and time is required (Halder and Azad, 2019). Biomass conversion technologies have their specifications and convert a specific composition to produce various forms of biofuels. To produce biofuels more efficiently and cost-effectively, a suitable process must be chosen. For commercial use, the production cost of an algae plant for biofuels must be lower than for petroleum fuels (Mutanda et al., 2020). The production of by-products is also dependent on the conversion process. However, currently, there is no efficient conversion process that converts algal biomass into biofuels in an environmentally friendly manner. Therefore, new strategies for techno-economic analysis, treatment designs, and production efficiency are essential (Halder et al., 2014). Therefore, additional exploration and growth in green technology are immediately needed to cost-effectively refine these fractions to maximize biomass utilization while improving the use of residual streams or by-products to develop more productive plans for commercial algae production facilities.

4. Bottlenecks and techno-economic analysis

Capital investment, operating costs, and revenues or sales are the three main components of an economic analysis of algal biofuel production. Therefore, the cost of algae biofuel production is determined by the sum of capital and operating costs minus revenues from all algae products (Sun et al., 2011). In addition to the biofuel, the sale of process by-products such as residual biomass, glycerol, and other high-value bioproducts such as protein and carbohydrates can generate significant revenue and reduce the overall cost of biofuel production. However, the techno-economic and life cycle analysis shows that microalgae-derived fuels are not cost-competitive with petrochemical fuels (Veeramuthu and Ngamcharussrivichai, 2020). Despite the potential of microalgae, several hurdles must be overcome before algae biofuel production can be commercialized. Culture conditions should be further improved to allow faster growth, higher biomass production, and the ability to compete for nutrients over fatty acids. Similarly, after biomass harvesting and dewatering, nutrient supply and water recycling are critical for the long-term production of microalgae biofuels (Faried et al., 2017). According to Chisti (2007) in an early economic study, the projected cost of algal biomass production is $2.95/kg for closed photobioreactors (PBR) and $3.80/kg for open raceway ponds. Similarly, Norsker et al. (2011) determined that the cost of algal biomass production in open ponds, horizontal tube PBRs, and flat-panel PBRs is €4.95, €4.15, and €5.96 per kg, respectively. The cost per liter of oil from biomass cultivated in closed PBRs was calculated to be $2.80/L, considering that the algal biomass contained 30% oil. The expected cost could be reduced even further to $0.72/L for algal biomass with 70% oil content (Chisti, 2007). The total cost of algal oil was estimated to be $3.46 based on a probabilistic TEA and Monte Carlo evaluation used for the algal oil extraction process and the dehydrated algae cycle in PBR culture and biodiesel production (hydroprocessing of extracted solvent lipid (HESL)). However, the lowest selling price (LSP) for biodiesel was $3.69 (Batan et al., 2016). The National Renewable Energy Laboratory (NREL) route, which included lipid extraction, fermentation, distillation, and hydrodeoxygenation, had an LSP of $0.96, while the UA route involved solvent extraction transesterification, and product purification for biodiesel synthesis, had an LSP of $2.32. There are significant differences in the LSP of the HESL and NREL pathways compared to the UA pathway. The UA route consists of traditional cultivation, harvesting, lipid production, and processing of the product, and lacks the addition of co-product credits. However, due to the integration of the production of various by-products, which in return generate revenue for the whole system, the economics of the NREL route are better (Dutta et al., 2016).

Although the cost of algal biodiesel in the United States was calculated to be $0.53–0.85/L (2012 value), the final cost of algal biodiesel was estimated by NREL to be in the range of $0.42-0.97/L (Nagarajan et al., 2013). A more recent study (Branco-Vieira et al., 2020) calculated the cost of algal oil biodiesel production to be €0.33/L and biomass production to be €2.01/kg in a 15.247-hectare plant. Total investment costs were reported to be $48/m^2 for open raceways and $66/m^2 for tubular PBRs at a scaled size of 100 ha (Norsker et al., 2011). Similarly, the economic approximation of microalgae biofuel production has also been published in other literature (Douskova et al., 2009; Heo et al., 2019; Singh and Gu, 2010; Stephens et al., 2010; Williams and Laurens, 2010). These studies confirmed that the cost of algae-based biofuels is currently higher than that of fossil fuels. Therefore, further advances in cultivation and downstream processing are needed to save energy and reduce costs. Integrating microalgae cultivation with wastewater treatment can reduce production costs by avoiding fertilizers, cutting costs on utilities and labor, increasing CO_2 efficiency, designing more productive PBR and raceway systems, and controlling depreciation, especially for harvesting equipment such as centrifuges. In addition, the

recovery of high-value by-products derived from leftover algal biomass, such as proteins, carbohydrates, rare earth metals, and medicinal compounds, can offset production costs and reduce the total cost of biofuel production.

5. Potential of microalgae for value-added bioproducts

Microalgae can produce bioactive molecules that are difficult to synthesize by chemical methods, such as antibiotics, subunit vaccines, monoclonal antibodies, hepatotoxic and neurotoxic substances, hormones, enzymes, and other pharmacological and therapeutic compounds (Rizwan et al., 2018). Table 2 shows the potential biomedical applications of several species of microalgae. Microalgae pigments have also been shown to offer health advantages, such as the prevention of cancer, heart disease, neurological problems, and eye diseases. Due to their unique characteristics, including fast growth and a simple, low-cost growth medium, microalgae are an ideal host for synthesizing recombinant proteins, and their post-translational modifications are more comparable to mammalian cells than to bacterial cells (Khavari et al., 2021; Ramana et al., 2017).

5.1. Antiviral substances

An ancient and proven practice in the manufacture of medicines is the use of active ingredients derived from natural resources. Several studies have shown that algae and cyanobacteria are rich in antiviral compounds. To date, unwanted viral and bacterial infections have developed, leading to adverse health consequences. Infections such as HIV and herpes require the use of drugs to effectively limit viral replication and provide an antiviral effect. Microalgae species are extensively used in the pharmaceutical industry due to the bioactivity of polysaccharides found in their cell walls (Ullah et al., 2019). The virucidal properties of the algae, combined with the enzymatic restriction ability, promote the growth of the syncytium, with the degree of sulfation being proportional to the anti-HIV purpose. Cyanovirin-N, a holistic chemical derived from Cyanobacteria, is a potent virucidal inhibitor that acts against HIV by interfering with the interaction of the viral glycoprotein gp120 with CD4 cells (Nowruzi et al., 2020).

Exopolysaccharides derived from *P. cruentum*, *Chlorella autotrophica*, and *Ellipsoidon* sp. have also been shown to have antiviral activity (Fabregas et al., 1999). According to Rizwan et al. (2018), the calcium spirulan sulfated polysaccharide derived from *S. platensis* inhibits virus ingression, demonstrating viral resistance activity, and helps in measles and herpes. The marine brown alga *Undaria pinnatifida* contains substantial fucoidan polysaccharides, mainly L-fucose and sulfate groups, with traces of galacturonic acid, xylose, galactose, and mannose. Fucoidan has been shown to promote bone growth by suppressing osteoblastic cell differentiation. In addition, the red alga *Gigartina skottsbergii* produces carrageenan, which is effective against enveloped and unenveloped viruses (Gopu and Selvam, 2020). Cell walls of other red macroalgae, such as *Kappaphycus alvarezii* and *Hypnea musciformis* (carrageenophytes), contain sulfated polysaccharides with antiviral characteristics comparable to those of Porphyridium (Bauer et al., 2021).

Many human diseases are caused by viruses (De Clercq, 2000); for example, severe acute respiratory syndrome (*SARS*) is caused by a novel coronavirus (SARS-CoV) (Carbone et al., 2021). Carrageenan and chitosan polysaccharides generated by different macroalgae have been found to have anti-SARS-CoV-2 properties (Drosten et al., 2003; Wu et al., 2020). Carrageenan is generated by the macroalga *Chondrius crispus* and is commercially used to combat IAV in a nasal spray solution (Bisolviral®), as well as approved as a food additive (GRAS). Great attention has been paid to SARS-CoV-2 due to the severity and contagiousness of the etiological agent (X. Chen et al., 2020; Fields et al., 2020). Sulfated polysaccharides found in marine algae have been shown to inhibit the growth of enveloped viruses. Several substances

Table 2
Different microalgae species have the potential to be used in biomedicinal applications (Morais Junior et al., 2020).

Microalgae	Component	Application	Reference
Chlorella zofingiensis, *Scenedesmus* sp. DHS, Selenastraceae sp. B10, *Pectinodesmus* sp. F13, *Chlorella* sp.	• Ester-astaxanthin • Astaxanthin • Glucan • β-Carotene • Lutein • Canthaxanthin	• Lowering the level of lipids in the blood • Increase the hemoglobin concentrations in the blood • During starvation, act as hypocholesterolemic and hepatoprotective agents • Enhances immunological response • Ethionine intoxication • Reduced blood sugar levels	(Barrow and Shahidi, 2007; Borowitzka and Borowitzka, 1988; Gong and Huang, 2020; Varfolomeev and Wasserman, 2011)
Dunaliella salina, *Chlorella* sp., *Nannochloropsis oculate*, *Dunaliella salina* CCAP 19/41, *Tetraselmis* sp. CTP4	• Glutathione • β-Carotene • Cryptoxanthin • Zeaxanthin • α-Carotene • Lutein • Canthaxanthin	• Anticancer activity • Protect eye cells • Antihypertensive • Antioxidant activity • Free radical neutralization • Broncholytic • Analgesic drug • AntiParkinson's disease	(Archer et al., 2019; Bhosale and Bernstein, 2005; Gonçalves et al., 2019; Gong and Huang, 2020; Leya et al., 2009; Schüler et al., 2020; Spolaore et al., 2006; Villar et al., 1992; Weinrich et al., 2019)
Arthrospira platensis, *Tetraselmis suecica*, *Dunaliella tertiolecta*, *Tetraselmis* sp., *Chlamydomonas reinharditii*	• Polyunsaturated fatty acids (PUFA) • Phycocyanin (colorant) • β-Carotene • γ-Linolenic acid (GLA) • Vitamin B1 • Vitamin B12 • Leucine • Provitamin A • Vitamin K • Isoleucinevaline	• Antioxidant • Immune system enhancement • Anti-inflammatory • Increase plasminogen activation factor production • Defend against viral infections • Lowering cholesterol levels • Prevent rheumatoid arthritis • Anti-tumor • Diabetes prevention • Reduce the risk of schizophrenia	(Andrade et al., 2018; Becker, 2007; Belay et al., 1993; Borowitzka and Borowitzka, 1988; Mobin and Alam, 2017; Patil et al., 2008; Richmond, 2008; Sajilata et al., 2008; Sathasivam et al., 2019; Spolaore et al., 2006)

(such as lectin, carrageenan, ulvans, and fucoidans from red, green, and brown algae) can act as biotherapeutic agents in the prevention and treatment of SARS-CoV disease (Pereira and Critchley, 2020). Dieckol is a phlorotannin isolated from the brown alga Ecklonia cava that has been shown to suppress SARS-CoV, 3CLpro trans/cis-cleavage in a dose-dependent and competitive manner without toxicity (Park et al., 2013).

5.2. Antibacterial substances

The microalgae component significantly reduces the pathogenic growth of bacteria in the water, preventing the development of bacterial infection. Significant reduction in *Escherichia coli* and *Salmonella typhimurium* growth is observed with the application of laminarin polysaccharide specifically derived from brown algae *Laminaria hyperborean* and *Ascophyllum nodosum* (Usman et al., 2017). Antibacterial activity against both Gram-negative and Gram-positive bacteria has been detected in extracts of *Chlamydomonas pyrenoidosa* and *C. vulgaris* (Rizwan et al., 2018). The qualitative composition of the sulfated functionality promotes remarkable biological properties. In addition, sulfated extracellular polysaccharides extracted from *Porphyridium cruentum* (de Jesus Raposo et al., 2014) and *Chlamydomonas* sp. (Sun et al., 2017) have been reported to have antibacterial activity, possibly related to their ability to form biofilms, which prevents adhesion and colonization of microorganisms on the surface of polysaccharides (Bernal and Llamas, 2012; Guzman-Murillo and Ascencio, 2000).

Due to the great diversity of microalgae species, only a few species exhibit pronounced antimicrobial properties. According to Rizzo et al. (2017), polysaccharides isolated from the green alga *Ulva fasciiata* and the brown alga *Dictyopteris polypodioides* were tested for antitoxin activity against *Aeromonas salmonicida* and *Vibrio alginolyticus*, both of which are neurotoxic. Another important marine red alga *Amphiroa rigida*, is commonly found along the southern coast of Tamil Nadu, India. Polysaccharide mediated by *Amphiroa rigida* is known for its excellent antibacterial activity against *Salmonella typhimurium* (Gopu and Selvam, 2020). The specificity of bacterial resistance could be attributed to several parameters, including temperature, pH of the culture medium, and variations in light intensity and quality, which affect metabolite synthesis and homeostasis in the human body (Rizwan et al., 2018).

5.3. Drug delivery

Drug delivery systems were developed to deliver drugs or genes to specific cells, such as cancer cells. A defective or missing genome is often found in patients with genetic diseases. Given this, gene transfer using silica nanoparticles (NPs) is considered successful (Dolatabadi and de la Guardia, 2011). Since they transport drugs to a specific location in the body, innovative drug delivery systems can overcome the constraints of traditional pharmaceuticals (e.g., low solubility/stability and high toxicity) (Khavari et al., 2021). Today, the use of marine resources in biomedicine is emphasized (Chao et al., 2014). Diatom biotechnology has received much interest in the last decade as a promising field for developing and producing high-value molecules with medicinal uses (Gordon et al., 2009). As mentioned earlier, microalgae are a significant source of numerous polysaccharides such as alginate, carrageenan, laminarin, and fucoidan, which can be transformed into NPs and used to interact with biomolecules through hydrophilic groups on the surface (Shankar et al., 2016).

Diatoms are brown algae with an amorphous silica exoskeleton that are both easy to grow and expensive. Porous silica (SiO_2) NPs can be found in their fossils [diatomaceous earth (DE)/frustules]. Live diatoms and DE are both sources of silica NPs, with DE having a higher proportion of frustules (Maher et al., 2018; Santhanam et al., 2019). In addition, silica NPs treated with cationic reagents (e.g., cationic amino silanes) are used for gene delivery. Sulfated polysaccharides, which play an important role in drug delivery systems, have exceeded the potential for drug formation. The cell wall of marine algae is composed of three compounds: carrageenan (found in red algae), fucoidan (found in brown algae), and ulvan (found in green algae) (Cunha and Grenha, 2016). Carrageenan has gained attention in recent years because of its wide range of applications as an emulsifier, thickener, and stabilizer. Although fucoidan is widely used in agriculture and medical fields, its economic importance is limited. Despite its importance in agricultural and pharmaceutical activities, the contribution of *Ulvan* has not been fully explored (Pereira, 2018). The proclivity of these polysaccharides, particularly carrageenan, allows them to meet the requirements of a specific drug delivery system by fully supporting the adhesive properties, which makes them crucial in the formulation of nanoparticles, beads, and film implants (Cunha and Grenha, 2016). Polysaccharides have helped usher in a new era of biomedicine by advancing regenerative medicine, tissue engineering, and drug delivery techniques. The behavior of primary polyelectrolytes allows the adaptation of activities to achieve goals that activate structural capacity (Sarangi et al., 2019). Drug delivery for the chemotherapeutic aspect to the target tumor cell is specified using the nanoparticle cargo-carrying platform of the microalgae concept. The magnetic effect, together with the biocompatibility property in *Chlamydomonas reinhardtii* by photocleavage of peptides under UV light irradiation, directed the association of microalgae with cargo (Zhong et al., 2021). Thus, it provides targeted delivery of chemo drug delivery to the metastatic region.

5.4. Carotenoids

Carotenoids are known for their antioxidant qualities, which are often highlighted when discussing their biological activities. Carotenes (α and β Carotenes and Lycopene) and xanthophylls (lutein, astaxanthin, zeaxanthin, and fucoxanthin) are antioxidant and anti-inflammatory microalgae carotenoids with potential biological applications (Miyashita, 2009; Rodrigues et al., 2012b; Seon-Jin, 2003). This group of carotenoids can also serve numerous functions; for example, -carotene can serve as a precursor to vitamin A, while zeaxanthin or lutein form the macular pigment in the eye (Arunkumar et al., 2020; Britton, 2020; Eggersdorfer and Wyss, 2018). Carotenoids have been shown to reduce oxidative stress, which benefits cardiovascular health. In addition, they can help prevent obesity, diabetes, some cancers, and neurological complications (Bonet et al., 2020; Eggersdorfer and Wyss, 2018; Rowles III and Erdman Jr, 2020). Where chronic diseases are significantly associated with an increase in oxidative stress. In addition, the oxidation of biologically relevant molecules, such as lipids, proteins, and nucleic acids, occurs when there is a cellular physiological imbalance between free radicals and the endogenous antioxidant system. Therefore, oxidative damage such as lipid peroxidation, mitochondrial enlargement, mutagenesis effects, and post-translational alteration of proteins occurs (Barkia et al., 2019; Pisoschi et al., 2020).

Fucoxanthin is a pigment found in the chloroplasts of *Phaeophyta* that generally absorbs light in the green-yellow part of the visible spectrum, with a maximum at 510–525 nm, corresponding to a brown or brownish-green hue. The low-light/dark phase of the xanthophyll cycle produces fucoxanthin, which plays a vital role in light harvesting and photoprotection (Büchel, 2020). Numerous studies have shown that the ingestion of fucoxanthin can provide significant biological processes while providing therapeutic benefits for health problems. Fucoxanthin acts as an antioxidant and scavenger of reactive oxygen species (ROS), inflammation-associated disorders, and is an antibacterial carotenoid. It has been shown to help treat several chronic diseases, including heart disease, type 2 diabetes, high cholesterol, hypertension, obesity, osteoporosis, metabolic syndrome, liver disease, cancer, eye and bone health (Bae et al., 2020).

In addition, the body relies on dietary antioxidants such as carotenoids to eliminate reactive species and hydroperoxides, particularly by donating electrons (Rodrigues et al., 2012a) or by stimulating the

mechanisms of the endogenous antioxidant system (do Nascimento et al., 2020; Kaulmann and Bohn, 2014). In this regard, microalgae are believed to be an important source of compounds with potent antioxidant activity. Thus, -carotene and astaxanthin have been shown to act as stimulators of endogenous antioxidant defenses, reducing lipid peroxidation and avoiding oxidative stress by removing free radicals (Le Goff et al., 2019). Ultimately, most research studies that examined the antioxidant capabilities of microalgae have shown that this trend mainly leads to the reduction of lipid peroxidation. Carotenoids are essential for maintaining good eye health (Eggersdorfer and Wyss, 2018). The most important carotenoids in the eye are lutein (36%), zeaxanthin (18%), and meso-zeaxanthin (18%), collectively known as macular pigment (Arunkumar et al., 2020). As antioxidants and blue-light filters, macular carotenoids protect the macula from light-induced oxidative damage (Jabbehdari and Handa, 2020; M. Khalid et al., 2019). The presence of hydroxyl groups related to the terminal ionone rings in macular pigments, as well as their conjugated double bond arrangement, are directly related to their light-absorbing capabilities and influence antioxidant activity as well as anatomical location (Jabbehdari and Handa, 2020).

5.5. Bioactive lipid production

Microalgal lipids and fatty acids are important HVAC components that have attracted interest due to their high content of saturated and unsaturated lipid molecules and are often used as feedstocks for biofuels and biomedical applications (Koyande et al., 2019; Kumar and Singh, 2019; Pérez et al., 2021). However, the composition and content of lipids vary from species to species. Several species of microalgae contain lipids that constitute 30-70% of the weight of dry cell biomass (Hossain and Mahlia, 2019). Omega fatty acids ($\omega-3$ and $\omega-6$) have been shown to have numerous medical benefits. Several studies have shown that only a small percentage of microalgae accumulate omega fatty acids within their cells and can be increased by simulating environmental/nutritional stress (Mehariya et al., 2021). Microalgae produce essential fatty acids (EFAs), mainly long-chain PUFAs such as gamma-linolenic acid (GLA) (18:3 $\omega-6$), arachidonic acid (AA) (20:4 $\omega-6$), EPA (20:5 $\omega-3$) and DHA (22:6 $\omega-3$) (Borowitzka, 2013; Ratledge, 2010). PUFAs have an essential function in cellular and tissue metabolism, including membrane fluidity control, electron and oxygen transport, and temperature adaptation (Funk, 2001). Unsaturated fatty acids decrease the risk of heart disease and atherosclerosis by reducing lipid levels, such as cholesterol and triglycerides. Several $\omega-3$ EPA and DHA are very important and nutritious among all other PUFAs. DHA is a structural fatty acid that is essential for brain and eye development in newborns and has been shown to promote cardiovascular health in adults (Ahmmed et al., 2020; Kroes et al., 2003; Ryckebosch et al., 2014; Ward and Singh, 2005). Furthermore, DHA can withstand signal transmission across nerve cells and protect against loss of scaffolding protein and oxidative lipid degradation (Ghasemi Fard et al., 2019). Humans are unable to synthesize the $\omega-3$ fatty acids EPA and DHA, which are required for basal metabolism; they must be obtained through diet (Odjadjare et al., 2017). Antioxidant EPA is very helpful for the cardiovascular system. It can open the arteries, cure atherosclerosis, prevent certain severe bleeding, and treat hypertension. EPA can possibly be used to cure brain disorders such as schizophrenia; it may even have a therapeutic effect on certain malignancies. Porphyridium spp. can produce significant amounts of ARA and EPA under certain conditions. The maximum ARA production (211.47 mg/L) was reported for *Porphyridium*, the highest known ARA production (Jiao et al., 2018). However, GLA is a $\omega-6$ polyunsaturated fatty acid -6 that plays a critical role in the production of prostaglandins. Studies have indicated that GLA can help cure arthritis, heart disease, obesity, depression, schizophrenia Parkinson's disease, multiple sclerosis, and zinc insufficiency in the elderly (Kerby et al., 1987). Further studies should focus on the suitability and safety of microalgae-derived DHA/EPA in foods. Future advances could promote the production of microalgae for improved nutrition and human health.

6. Future research prospective

Algae represent a novel biofuel source that has the potential to replace fossil fuels in the future. Green algae have several advantages as a fuel source: (1) green algae contain a significant amount of lipids; (2) waste and wastewater resources can be used for general algal biofuels; (3) the use of algal biofuels can reduce CO_2 emissions in the atmosphere; and (4) biofuel production can provide many valuable products such as pigments, pharmaceutical compounds, fertilizers, agar, and lubricants. Given the importance of algae, global production of algal biomass has increased significantly over the past 50 years. However, the low economic viability of algal biofuels and the high cost of the production process have limited their commercial production. In addition, several challenges must be overcome before algal biofuel technology can compete with the petroleum industry. The design of the cultivation system, selection of strains, availability of space and water, sufficient light, utilization of CO_2, growth of the algae, uptake of nutrients, harvesting and drying of the biomass, extraction of the oil, and conversion into biofuels are just some of the important issues that need to be addressed. To this end, new strains of algae need to be explored and existing strains genetically improved to increase oil content and overall production, as well as improve their tolerance to pathogens and environmental stressors. Integrating algal fuel production processes with other technologies, particularly wastewater treatment, is a potential economic and cost-effective approach. Similarly, biofuel costs can be further reduced through the co-extraction of valuable byproducts. Advances in green technology based on microalgae can benefit the environment while providing other value-added products, such as biofertilizers and animal feed. However, additional efforts and research are needed to properly link green technology with microalgae. There are great opportunities for researchers in this field to explore additional potential applications of microalgal biomass and to develop innovative value-added products from microalgae that can generate revenue. Given the interest it has generated among scientists, governments, and industry, it is expected that algal biofuel production technology will eventually become commercially viable.

7. Conclusions

This review article discusses various aspects of microalgae, including their use in integrated wastewater treatment, bioenergy production, and the production of high-value bioproducts. The integration of wastewater treatment with microalgae extraction has attracted attention due to its environmental sustainability. The integrated microalgae biorefinery system has the potential to significantly reduce wastewater treatment costs while providing long-term solutions for a circular economy and greener industrial practices. Aspects such as potential biofuels produced from microalgal biomass and the use of microalgal biomass as an energy source are also discussed. Recent technological advances have improved the economic viability of third-generation biofuel production while reducing overall process costs. Techno-economic assessment could also be used to design a low-cost microalgae biorefinery that manages wastewater treatment in a circular economy. In summary, more integrated methods for wastewater treatment, microalgae culture, bioenergy production, and other high-value bioproducts are critical to improve industrial feasibility and profitability.

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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