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MICROALGAE WASTEWATER TREATMENT AND INTEGRATED PRODUCTION OF HIGH-VALUE-ADDED PRODUCTS FOR ENVIRONMENTAL SUSTAINABILITY

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Abstract: Microalgae are a potential bioremediation for wastewater treatment due to their capacity to exploit nutrients found in wastewater. Extensive research has been done to improve microalgae-mediated wastewater treatment using a variety of technologies, including membranes, ponding systems, chemical methods, and artificial wetlands. However, addressing the challenges related to cost and energy demands in microalgae cultivation is crucial for wider adoption. Hence, this review highlights recent advancements in microalgae-mediated technology, with a focus on integrating water treatment and recovering valuable products. It discusses the development of microalgae-mediated nanomaterials for efficient water treatment and explores the application of microalgae-based biosensors to provide real-time insights for improved environmental management. Furthermore, the sustainable use of microalgae technology in constructed wetlands for water treatment is emphasised. The review also rigorously investigates the challenges and prospects associated with microalgae cultivation in wastewater to produce valuable products. This comprehensive information contributes to advancing sustainable environmental management practices, paving the way for a greener and more resilient future.

Keywords: Microalgae, wastewater treatment, value-added product, environmental, sustainability.

Introduction

Microalgae, with their remarkable environmental benefits have emerged as a promising alternative feedstock for the energy industry. Their utilisation in biofuel production and wastewater treatment has gained significant attention due to their ability to address pressing environmental concerns (Purba *et al*., 2024). This review article focuses on the trend of microalgae-based wastewater treatment and the integrated production of high-value products while highlighting the pivotal role of microalgae in promoting environmental sustainability.

Microalgae, unicellular photosynthetic microorganisms, possess several advantages that make them ideal for sustainable energy solutions. Notably, microalgae have been identified as a promising and environmentally friendly alternative feedstock for the energy industry. Their cultivation does not rely on arable land, which helps to avoid competition with food crops, thus, minimising the environmental impact on food production (Ahmad & Ashraf, 2023). Additionally, microalgae exhibit rapid growth rates and high photosynthetic efficiency, making them efficient converters of solar energy into biomass. This unicellular photosynthetic microorganism can be found in a variety of forms, ranging from single-cell picoplankton between 0.2 µm and 2 µm to filamentous forms with sizes reaching 100 µm or larger. Microalgae are a diverse group of marine and freshwater microorganisms capable of synthesising proteins, carbohydrates, and lipids through photosynthesis (Ravindran *et al*., 2016). Because of their size, microalgae are categorised as either prokaryotic (cyanobacteria) or eukaryotic (green and red microalgae and diatoms) (Temraleeva *et al*., 2023). By harnessing the inherent environmental advantages of microalgae, their cultivation and utilisation offer a sustainable approach to meeting energy demands while mitigating the ecological impact.

Microalgae cultivation has been applied to a wide range of applications, including human and animal nutrition, bioenergy generation, food and beverage production, and intriguing applications in the pharmaceutical and cosmetics industries. In addition to their versatile applications, microalgae also play an important role in promoting environmental sustainability through biofuel production and wastewater bioremediation. These living cell factories can generate microalgal oil, which can then be used as a feedstock for biodiesel production. Their microalgal oil is rich in triacylglycerols, which can be converted into biodiesel through transesterification. The percentage of triacylglycerols varies depending on the species and growth conditions. According to Pandey *et al*. (2024), triacylglycerols constitute 20%-50% of the cell dry weight. In addition, the saturated and unsaturated fatty acids present in microbial oil vary between 20% and 70% (Zhou *et al*., 2022), influenced by a combination of genetic and environmental factors (Morales *et al*., 2021).

Additionally, the lipid content extracted from the microalgae with microbial oil (Zhou *et al*., 2022) varies according to different species. Several freshwater and marine microalgae species such as *Botryococcus braunii* (Murata *et al*., 2015; Jackson *et al*., 2020; Oni *et al*., 2021), *Chlorella* spp. (Dash & Banerjee 2017; Zhao *et al*., 2018; Trivedi *et al*., 2019), and *Nannochloropsis* spp. (He *et al*., 2020; Schambach *et al*., 2020; Abimbola *et al*., 2021). It was demonstrated that the potential to produce lipid contents of up to 70%. The extracted microalgal oil, primarily composed of neutral lipids, contains a high content of unsaturated fatty acids, with lipid content varying significantly depending on microalgae species, environmental conditions, and other factors (Zhou *et al*., 2022).

According to Morales *et al*. (2021), lipid accumulation is higher in the stationary phase than in the exponential phase. During the exponential phase, carbon is allocated for cell growth and division, whereas in the stationary phase, nutrient limitations slow growth and redirect energy toward lipid production. Furthermore, the growth medium composition, culture mode (phototrophic, heterotrophic, or mixotrophic), pH, temperature, and light intensity are other factors that influence the lipid profile of microalgae (Sivaramakrishnan & Incharoensakdi, 2019).

Microalgae are remarkably adaptable to a wide range of environments, making them easily culturable both indoors and outdoors. Moreover, microalgae have a shorter life cycle than plants, which enables them to produce up to 5 to 10 times more biomass and up to 15 to 300 times more lipids than terrestrial crops (Jayaseelan *et al*., 2021). Lipid accumulation in microalgae is triggered as a response to environmental stressors such as limited nitrogen, phosphate, or other nutrients in the growth medium, further highlighting their environmental adaptability.

Wastewater treatment poses significant environmental challenges due to its inefficiency in removing toxicants, heavy metals, nitrogen, and phosphorus while also complying with stringent effluent standards. However, microalgae have emerged as a promising technology for addressing these issues by effectively removing nutrients and organic pollutants from wastewater. Al-Jabri *et al*. (2021) have highlighted that wastewater effluents are rich in nitrogen, phosphorus, and other trace elements, which can serve as valuable nutritional sources for heterotrophic or mixotrophic microalgal growth and cultivation.

However, certain microalgae species such as *Chlorella vulgaris* can grow under both autotrophic and heterotrophic conditions in wastewater containing inorganic and organic chemical compounds (Zabochnicka, 2022). Mubashar *et al*. (2024) reported that high-rate nutrient recovery was achieved through the integration of autotrophic (light-dependent) and heterotrophic (organic carbon-dependent) growth. The synergistic activities resulting from the internal cycling of oxygen (O_2) from autotrophic photosynthesis and carbon dioxide (CO_2) from heterotrophic respiration further enhance resource utilisation (Liu *et al*., 2023).

When subjected to stress conditions such as low nitrogen or phosphorus levels, microalgae can accumulate up to 64% lipids based on dry cell weight (Griffiths & Harrison, 2009). Fatemeh *et al*. (2021) reported that microalgae-based bioremediation had shown an effective removal of organic compounds, carbon, nitrogen, and phosphorus in wastewater to a safe level before releasing into waterways. The Malaysian Department of Environment (DOE) emphasises that the safe level of organic compounds such as Biochemical Oxygen Demand (BOD), prior to discharge into waterways should be reduced from 100 mg L^{-1} to 20 mg L^{-1} (Lokman *et al*., 2020).

Serrà *et al*. (2020) found that microalgae can remove heavy metals from wastewater, even under highly acidic or alkaline conditions with pH levels ranging from 2 to 10. *Chlamydomonas acidophila* and *Chlorella vulgaris* are among the species that can effectively remove toxic heavy metals due to their physiological characteristics, including high resistance to acidic conditions (Díaz *et al*., 2020) and tolerance to high alkalinity, with pH levels up to 10.5 (Katırcıoğlu *et al*., 2023). Furthermore, Nie *et al*. (2020) reported that microalgae can also remove pharmaceutical chemicals and pesticides from industrial and agricultural wastewaters, further highlighting their potential as an effective and sustainable wastewater treatment technology with significant environmental benefits.

Diverse microalgae species exhibit the remarkable ability to remove nitrogen, phosphorus, and other pollutants from various type of wastewaters, including sewage, aquaculture effluents, agricultural runoff, and industrial effluents, thereby addressing significant environmental concerns. Extensive research has highlighted the economic potential and sustainability of using microalgae grown via wastewater treatment as a valuable biofuel feedstock (Collotta *et al*., 2018; Silva *et al*., 2021). The cultivation of microalgae in wastewater offers dual benefits by not only producing valuable biomass for biofuel production but also reducing the biochemical and chemical oxygen demands of the wastewater. For example, a study has shown that *Parachlorella kessleri* grown in municipal wastewater was highly productive (56 mg/L/day) and yielded a high lipid content (38%) while also removing 99% and 82% of nitrogen and phosphorus compounds, respectively (Aketo *et al*., 2020).

Adopting an integrated approach that utilises microalgae for wastewater treatment presents several environmental advantages, including a reduction in freshwater demand by up to 90% and reduced fertiliser costs. This is particularly advantageous because traditional microalgae cultivation methods typically require a large amount of water, about 3,726 kg of water per kg of microalgal fuel produced (Yang *et al*., 2011). Implementing microalgae-based wastewater treatment techniques not only addresses the issue of wastewater pollution but also contributes to conserving freshwater resources and reducing the reliance on conventional fertilisers, promoting a more sustainable and environmentally friendly approach to biofuel production.

Microalgae have also been explored as a source of nanomaterials for enhancing the effectiveness of wastewater treatment. Microalgae can produce biological compounds that serve as reducing agents for synthesising inorganic nanoparticles to remove heavy metals from wastewater (Chan *et al*., 2022). The ability of microalgae to survive in diverse and extreme conditions makes them a potential bioagent for the effective management of heavy metal pollution. Microalgae have also been investigated for their potential as biosensors for water quality monitoring. According to Allouzi *et al*. (2022), microalgae possess an exceptional sensitivity to changes in metabolic activity resulting from exposure to toxins. These changes can be converted into electrical or optical signals within biosensors that rely on microalgae, making them useful for detecting and monitoring the presence of toxins (Chong *et al*., 2021). This innovative approach not only utilises the unique characteristics of microalgae but also promotes sustainable and efficient water quality management practices, safeguarding ecosystems, and human health.

The metabolic processes of microalgae are significantly influenced by toxic substances such as those found in industrial, domestic, agricultural wastewater, and food waste sources. The Tricarboxylic Acid (TCA) cycle regulates carbon and nitrogen flow, integrating the metabolism of carbohydrates, proteins, and lipids in photosynthetic microalgae (Chen & Wang, 2021). Metabolic processes also include intercellular interactions, vesicle transport, transcriptional regulation, signal transduction, and translational control (Dolganyuk *et al*., 2020).

Heavy metal contamination disrupts these processes, prompting microalgae to utilise detoxification mechanisms such as biosorption and bioaccumulation, supported by specific transporters (Chakravorty *et al*., 2023). Additionally, species like *Chlorella marina*, *Tetraselmis suecica*, and *Picochlorum maculatum* efficiently remove inorganic nitrogen compounds (NH_3-N and NO_2-N) from aquaculture wastewater (Meril *et al*., 2022). However, heavy metals like mercury, cadmium, lead, and chromium are highly toxic and disrupt metabolic functions in microalgae, potentially causing toxicity, mutagenesis, and allergenicity (Mahlangu *et al*., 2024).

In addition to biofuel, the cultivation of microalgae offers a plethora of opportunities for generating a wide range of high-value-added products. These versatile microorganisms have demonstrated their potential in producing valuable compounds such as carotenoids, pigments, proteins, and antioxidant compounds. Some microalgae species such as *Scenedesmus almeriensis*, *Nannocholoropsis* sp, and *Haematococcus pluvialis* are particularly rich in carotenoids, including beta-carotene, astaxanthin, and lutein (Molino *et al*., 2018). Microalgae serve as a sustainable and efficient source for these valuable products, offering a promising alternative to synthetic or animal-derived sources.

This comprehensive review article thoroughly evaluates the immense potential of microalgae-based technology in the field of wastewater treatment. It delves into the various aspects of this innovative approach, including its challenges and prospects for scaling up from small-scale laboratory experiments to large-scale commercial production. In addition to wastewater treatment, the article explores the broader applications of microalgae cultivation, specifically in the production of biofuels and other value-added products.

It examines the integrated approach that combines wastewater treatment with the generation of valuable products or compounds, highlighting the multifaceted benefits and synergies that can be achieved through this dual-purpose strategy. By considering the integrated production of biofuels and value-added products, the article showcases the immense potential of microalgae cultivation as a sustainable and environmentally friendly approach with significant economic and environmental benefits.

Microalgae Cultivation in Various Waste Resources

Microalgae cultivation in various waste sources is an emerging field that has garnered significant attention in recent years. This approach entails using various waste sources as a nutrient source for growing microalgae such as municipal or industrial wastewater, agricultural runoff, or landfill leachate (Table 1). The cultivation of microalgae in waste media offers several advantages, including the ability to produce value-added products for various applications while simultaneously reducing pollution.

Microalgae have been cultivated using various water sources and processing strategies. Koçer *et al*. (2023) have investigated the hydrothermal carbonisation process of water in different concentrations (0.25%-2%) for microalgae cultivation and nutrient recycling. The optimal growth rate and doubling time were determined to be 0.16 day^{-1} and 4.44 days, respectively. The resultant carbohydrate, lipid, and protein contents of microalgae varied between 32.25% and 35.03%, 12.71% and 14.33%, and 35.36% and 41.22%, respectively. The study by Tarhan *et al*. (2021) showed that the dilution rate of the process water affects the growth rate of microalgae. They studied the potential use of process water from the hydrothermal carbonisation of olive and orange pomace as a nutrient source for microalgae cultivation. The process waters were diluted with distilled water at 50×, 100×, 200×, and 400× dilutions and used to cultivate *Chlorella minutissima* and *Botryococcus braunii* microalgae. The results showed that lower dilution levels resulted in higher growth rates and shorter doubling times for both microalgae species.

In Palm Oil Mill Effluent (POME), the microalgae *Ankistrodesmus fusiformis* and the yeast *Rhodotorula toruloides* were co-cultivated, according to Justine *et al*. (2023). In this study, under optimal conditions, which included a POME concentration of 59.88% and a yeast-to-microalgae ratio of 9:25 during the 18-day growth period, the COD was reduced by 72.27% and produced 0.012 mg/L/d lipid productivity. Furthermore, the efficacy of various microalgae species in treating wastewater varies and if they are not brought down to an acceptable level, they may become toxic (Jasni *et al*., 2021). According to Ahmed *et al*. (2022), high wastewater concentrations

Table 1: Cultivation of microalgae from various waste substrates for pollutant removal

Source of Wastewater	Microalgae	Pollutant Removal	References
Hydrothermal Carbonisation (HTC) process water	*Chlorella sorokiniana*	-	(Koçer *et al*., 2023)
Orange and olive pomace water	*Chlorella multissima*, *Botryococcus braunii*	-	(Tarhan *et al*., 2021)
Cattle farm wastewater	*Chlorella* sp., *Kirchneriella obesa*	Nitrogen (95%), phosphorus (99%), and COD (82%)	(Wang *et al*., 2022a)
Food waste digestate	*Chlorella sorokiniana*	Ammoniacal (> 70%)	(Barzee *et al*., 2022)
Dairy waste streams	Mixotrohphic microalgae	Organic (92.6%), inorganic (48.5%-98.4%)	(Patel *et al*., 2020)
Palm oil mill effluent	*Rhodotorula toruloides*, *Ankistrodesmus falcatus*	COD (72.27%)	(Justine *et al*., 2023)
Starch wastewater	Microalgae and fungi	COD (92.08%), total nitrogen (83.56%), total phosphorus (96.58%)	(Wang *et al*., 2022b)

may hinder the growth of microalgae, for example, the *Chlorella vulgaris* absorbs CO_2 and other carbon sources, raising the pH of the growth media. Hence, the increase in pH could negatively impact the growth of *C. vulgaris*.

Cheaper media have been explored as a cost-effective alternative to traditional standard media for microalgae cultivation. It is defined as growth media that utilise nutrient-rich, low-cost resources such as wastewater to reduce cultivation expenses. Wastewater is particularly considered a cheaper medium due to its rich nutrient content, which efficiently promotes microalgae growth (Justine *et al*., 2023). Cultivating microalgae in wastewater sources can reduce costs and lead to the bioremediation of waste sources. These processes are environmentally friendly and cost-effective. According to Justine *et al*. (2023), the microalgae, *Ankistrodesmus fusiformis* were co-cultivated with yeast, *Rhodotorula toruloides*, in Palm Oil Mill Effluent (POME). In this study, the COD was reduced by 72.27% and yielded 0.012 mg/L/d lipid productivity under ideal conditions, which included a POME concentration of 59.88%, a yeast-to-microalgae ratio of 9:25 at an 18-day growth period.

Aron *et al*. (2021) have comprehensively reviewed the cultivation of microalgae in wastewater sources and the promising technologies to convert them into useful bioproducts. The researchers studied the cultivation of two types of microalgae, *Chlorella sp*. (FACHB-8) and *Kirchneriella obese* (FACHB-2104), using unsterilised Cattle Farm Wastewater (CFW) filtered through corn stover as growth media (Wang *et al*., 2022b).

The study found that corn stover filtration effectively reduced the turbidity and suspended solids of CFW. Under optimal conditions (3:6 ratio of CFW/algae medium and 26-400 μmol photons m^{-2} s^{-1}), maximal yields of FACHB-8 and FACHB-2104 were 1.26 and 1.22 g/L, respectively. The removal efficiencies of nitrogen, phosphorus, and chemical oxygen demand exceeded 95%, 99%, and 82%, respectively.

Meanwhile, Barzee *et al*. (2022) cultivated microalgae using food waste digested in a pilot-scale outdoor system with extensive water recycling. Supernatant liquid from algae harvesting was recycled five times, decreasing coagulant loading requirements, and eliminating liquid non-product discharge and freshwater inputs. High removals of ammoniacal-N (> 70%) were achieved, but water recycling led to ion accumulation. The repeated batch harvesting and liquid recycling strategy will inevitably result in some materials building up in the reactors. The use of $FeCl^3$ as a harvesting aid increases the likelihood that Cl^- ions will accumulate in the reactor. As a result, the concentration of Cl^- is extremely high for the cultivation of algae, and the increase in concentration over time can be linked to a number of changes in the microalgae, including elevated lipid production or decreased chlorophyll production. Their study demonstrated promising high removals of ammoniacal-N for more than > 70%.

Patel *et al*. (2020), on the other hand, investigated the pre-treatment of whey from dairy waste streams to make it suitable for algae cultivation. They employed a combination of acid hydrolysis, chemical flocculation, and struvite formation to remove unwanted substances such as polymers, solid fractions, and overloads of organic and inorganic compounds. A 40% concentration of pretreated whey yielded the highest biomass and lipid production, at 4.54 g/L and 1.80 g/L, respectively, with daily productivities of 0.50 g/L/day and 0.20 g/L/day.

Wang *et al*. (2022) studied a one-step co-cultivation and flocculation of microalgae with filamentous fungi in starch wastewater. The microalgae and fungi were mixed and incubated under controlled conditions of light and temperature. After the co-cultivation period, the microalgae and fungi were separated from the wastewater through flocculation, allowing the biomass to settle out of the solution. The synergistic effects between them greatly improved the removal efficiency of COD, TN, and TP up to 92.08%, 83.56%, and 96.58%, respectively.

Aspergillus oryzae achieved higher biomass (2.23 g/L) than *Chlorella pyrenoidosa* (1.79 g/L) by utilising organic polymers in PSW. The algae-fungal consortia outperformed both in biomass production (3.53 g/L), approximately 1.58 and 2.04 times higher than *A. oryzae* and *C. pyrenoidosa*, respectively. While *C. pyrenoidosa* had the highest protein content (58.13%) and *A. oryzae* had 43.20%, the consortia exhibited balanced protein (54.30%) and lipid (28.10%) levels, with overall yields enhanced by increased biomass production.

Despite the potential benefits of microalgae cultivation in waste sources, there are challenges associated with this approach, including the need for careful monitoring and management to maintain optimal growth conditions and minimise contamination. A comprehensive review of the progress and challenges of contaminant removal from wastewater using microalgae biomass has been reported by Ahmed *et al*. (2022). The review compares conventional and microalgae-based systems for treating wastewater, examining their efficiency, characteristics of the wastewater, and mechanisms for removing nutrients and heavy metals.

Additionally, the economic feasibility of large-scale microalgae cultivation in waste sources requires further investigation. Velásquez-Orta *et al*. (2024) demonstrated the significant economic advantage of using wastewater as a replacement for standard cultivation media in microalgae production. This substitution reduced production costs from $2.71/kg biomass to $0.73/kg biomass, highlighting its potential to drastically lower cultivation expenses. However, large-scale cultivation, especially in open systems often encounters challenges such as microbial contamination due to non-sterile conditions. These conditions limit the strains to only those that are fast-growing, resilient, and tolerant of harsh environments (Srimongkol *et al*., 2022).

Avinash *et al*. (2020) have analysed in their study the limiting factors for large-scale microalgal cultivation as a promising future for the renewable and sustainable biofuel industry. Various operational and environmental factors were identified, resulting in inefficient cultivation of microalgae. For large-scale microalgal cultivation, microalgae with high growth rates, high lipid content, and high productivity are highly demanded. The researchers highlighted the importance of addressing environmental resource limitations such as a shortage of carbon sources, optimal light intensity, temperature, essential nutrients, and pH or salinity changes, during culturing. Cost-effective technology can help improve biomass concentration and productivity.

To conclude, the cultivation of microalgae in wastewater sources presents a promising avenue for cost-effective and environmentally friendly nutrient recycling and bioremediation. The studies reviewed in this article highlight the potential of using wastewater sources for microalgae cultivation and the development of innovative technologies for the conversion of microalgae into valuable bioproducts.

Advances in Microalgae Cultivation in Wastewater

Microalgae for Nanomaterials in Wastewater Treatment

In recent years, microalgae have gained attention for their low-cost and environmentally friendly applications in synthesising metallic nanomaterials, particularly for wastewater treatment. Studies have shown the potential of microalgae in producing nanoparticles and quantum dots for biosensing and detecting environmental pollutants. Chin *et al*. (2023) reviewed the sustainable and economical use of microalgae for nanoparticle synthesis in wastewater treatment, focusing on the biogenesis of nanoparticles and their applications in biosensing and pollution detection.

However, Xiong *et al*. (2022) highlighted the environmental risks associated with using engineered nanoparticles in microalgal wastewater treatment. Similarly, Song *et al*. (2022) explored the mechanisms, applications,

Table 2: Biosynthesis methods and sources for nanoparticle production using various biological and chemical techniques

Type of Biosynthesis	Type of Nanoparticle	Method	References
Microwave-assisted synthesis	Metal nanoparticles	Application of heat (heating mantles)	(Rahman *et al*., 2020)
	Silver nanoparticles (AgNPs)	Microwave-assisted synthesis of AgNPs using glucose and starch	
Carbon-based nanomaterials	Carbon nanotubes (CNTs)	Hydrothermal treatment of soy milk	
Algae-mediated	-	-	
Cyanobacterial strains (*Anabaena flos-aquae*, *Calothrix pulvinata*, and *Leptolyngbya foveolarum*)	-	Intracellular synthesis	
Extracted biomolecules (*Chlorella vulgaris*)	AuNPs	High-performance liquid chromatography	
Cell- free supernatant (algal cells)	Nanoparticles (NPs)	Centrifugation	
Whole cells (*Euglena gracilis*, *Euglena intermedia*, *Navicula minima*)	Nanoparticles (NPs)	Centrifugation/filtration or simple washing of the cells	
Microalgae (*Klebsormidium flaccidum* and *Cosmarium impressulum*)	Au (gold) nanoparticles	Combination of structural, chemical, and biological analyses	(Dahoumane *et al*., 2012)
Cyanobacteria and microalgae	Silver nanoparticles (AgNPs)	Adding live and cleaned cyanobacteria and microalgae biomass to the AgNO3 solution, by incorporating AgNO3 into a culture liquid devoid of cells	(Patel *et al*., 2015)
Microalgae (*Chlorella vulgaris*)	-	Incubation in gold chloride and harvested through centrifugation	(Luangpipat *et al*., 2011)
Microalgae (*Spirulina platensis*)	Silver nanoparticles (AgNPs)	UV-visible spectroscopy, transmission electron microscopy, and X-Ray Diffraction (XRD)	(Mahdieh *et al*., 2012)
Microalgae (*Desmodesmus abundans*)	Silver nanoparticles (AgNPs)	Acclimated to low and high CO_2	(Mora-Godínez *et al*., 2022)
Microalgae (*Coelastrella aeroterrestrica* BA_Chlo4)	Hexagonal silver nanoparticles (AgNPs)	-	(Hamida *et al*., 2022)
Microalgae nano-bio hybrid	ZnO nanoparticle	-	(Vasistha *et al*., 2021)

and challenges of microalgal-based wastewater treatment, emphasising the need to optimise key process conditions such as pH, temperature, and light intensity to improve efficiency. Table 2 provides an overview of biosynthesis methods and sources for nanoparticle production using various biological and chemical techniques.

The study of algae-mediated biosynthesis of nanometals has been termed phytonanotechnology. The presence of hydrophilic surface groups, including sulphate, carboxyl, and hydroxyl, on nanomaterials mediated by algae provides them with distinctive potential applications (Rahman *et al*., 2020). Several important process parameters can affect the biosynthesis process, including pH, temperature, incubation time, precursor concentration, and light intensity. According to Agarwal *et al*. (2019), microalgal nanoparticles have become an innovative solution to circumvent issues associated with the microalgal culture system such as highly time-consuming, tedious tasks, harvesting of microalgae, and extensive use of arable land.

Various microalgal species have been utilised for the biosynthesis of nanomaterials, including *Haptophyta* sp, *Ochrophyta* sp*, Klebsormidium* sp, and *Cosmarium* sp (Merin *et al*., 2010; Dahoumane *et al*., 2012). Patel *et al*. (2015) screened cyanobacteria and microalgae for their ability to synthesise silver nanoparticles with antibacterial activity. The biosynthesis of silver nanoparticles (Ag-NPs) was carried out using two procedures: (i) By suspending the live and washed biomass of microalgae and cyanobacteria in the $AgNO_3$ solution and (ii) by adding $AgNO_3$ into a cell-free culture liquid. The resulting Ag-NPs were found to vary in shape and size, ranging between 13 nm and 31 nm, depending on the organism used.

Luangpipat *et al*. (2011) used live cells of the green microalga *Chlorella vulgaris* to produce gold nanoparticles. The algae cells were incubated with a solution of gold chloride and harvested through centrifugation, producing intracellular gold nanoparticles measuring 40 nm - 60 nm in diameter. The recovered gold concentration from the algal cells was 97% using a 1.4% Au concentration.

Meanwhile, Mahdieh *et al*. (2012) synthesised silver nanoparticles using *Spirulina platensis* in an aqueous system of silver ions. The resulting silver nanoparticles were characterised using UV-visible spectroscopy, transmission electron microscopy, and X-Ray Diffraction (XRD). Mora-Godínez *et al*. (2022) synthesised silver nanoparticles (AgNPs) using *Desmodesmus abundans* green algae acclimated at low and high CO_2. Meanwhile, a novel microalgae strain, *Coelastrella aeroterrestrica* BA_Chlo4 has been utilised to synthesise hexagonal silver nanoparticles with a small size of 14.5 nm (Hamida *et al*., 2022).

Additionally, microalgae nano-bio hybrid has also opened a new paradigm for efficient wastewater treatment and biofuel production. The influence of microalgae-ZnO nanoparticle association for the removal of nutrients and improved biodiesel production has been studied by Vasistha *et al*. (2021)—the isolated microalga *Chlorosarcinopsis* sp. MAS04 was cultured in primary treated wastewater (PTWW) and secondary treated wastewater (STWW) incorporated with Zinc Oxide nanoparticles (ZnO NPs). The maximum biomass achieved was 3.43 g/L and 2.14 g/L in the presence of PTWW and STWW, respectively. Furthermore, the presence of PTWW and STWW resulted in a 1.9-fold and 1.5-fold increase in cellular lipids, respectively, with a higher quantity of Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA).

Microalgae are promising resources for nanomaterials synthesis as a low-cost, eco-friendly, and effective alternative to traditional wastewater treatment methods. Nevertheless, there are still some challenges that need to be addressed. Further research is needed to optimise the process conditions and evaluate the environmental risks associated with the use of nanoparticles in wastewater treatment.

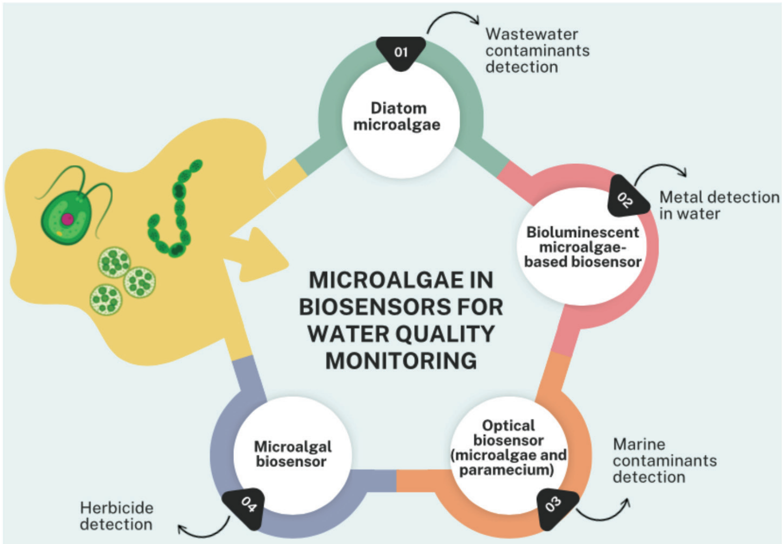

Figure 1: Roles of microalgae in biosensors for water quality monitoring

Microalgae in Biosensors for Water Quality Monitoring

Microalgae have recently emerged as promising biosensors for monitoring water quality due to their unique qualities such as high sensitivity, low cost, and ease of use. Khan *et al*. (2021) highlighted the potential of diatom microalgae as intelligent nanocontainers for biosensing wastewater contaminants. Diatoms are robust microalgae that protect themselves against invaders and maintain their internal temperature by encasing biomolecules in their silica structure. The study emphasised the versatility of diatom microalgae, which can be engineered for biosensing various pollutants, including heavy metals, pesticides, and organic compounds. The roles of microalgae in biosensors for water quality monitoring are summarised in Figure 1.

Diatom microalgae have been used as smart nanocontainers for biosensing wastewater pollutants. As described in Khan *et al*. (2021) review, diatoms can remediate various pollutants such as heavy metals, dyes, and hydrocarbons detected in the wastewater. Diatoms possess a stunning nanoarchitecture that enables them to selectively bind to ligands and create a nanocomposite structure with various pollutants, either chemical or biological. These naturally occurring diatom nanomaterials are not only cost-effective but also highly sensitive compared to their artificially synthesised silica counterparts, making them a useful tool for efficiently removing toxic pollutants from wastewater.

Metal detection in water is critical for protecting human health, aquatic life, and environmental safety. Wong *et al*. (2018) have successfully developed a bioluminescent microalgae-based biosensor for metal detection in water. Microalgae and other photosynthetic cells respond to environmental metals through metabolic processes that electrical transducers can easily detect. The developed biosensor demonstrated high sensitivity to heavy metals (Cu, Pb, Cd) and light metals (Na, Al, Li) and could be used to screen drinking water quality, making it a promising tool for the environmental monitoring of heavy metal pollution. In addition, Roxby *et al*. (2020) designed an electrochemical microalgae sensor for metal ion detection that is enhanced by a nanocavity between Cu nanoparticles and the electrode. The biosensor achieved high selectivity and sensitivity for metal (cadmium, iron, chromium, and manganese) ion detection with a detection limit of 50 nM in 10 seconds. Because the device is microfluidic, it can be used both offline and online.

The escalation of human-caused pollution in marine ecosystems has rendered imperative the creation of cost-effective, versatile, and responsive early-warning mechanisms for the on-site detection of chemical pollutants. Turemis *et al*. (2018) developed a novel optical biosensor that uses the symbiotic relationship between microalgae and paramecium to detect relevant marine contaminants. The biosensor demonstrated high sensitivity and specificity for detecting marine pollutants such as heavy metals and pesticides. Han *et al*. (2019) developed a digital microfluidic diluter-based biosensor for monitoring marine pollution using microalgal motion analysis. The biosensor achieved high sensitivity for detecting pollutants such as toxic metals and phenol in seawater. Both technologies have potential applications in improving the monitoring and management of marine pollution.

Microalgae-based biosensors have also been employed for herbicide monitoring. Boron *et al*. (2020) developed a portable microalgal biosensor utilising reversible photosynthesis inhibition produced by herbicides on microalgae for on-site herbicide monitoring in rivers. The biosensor exhibits outstanding performance in river samples, with a detection limit of 0.11 μM and sustaining its integrity for at least five months, rendering it an ideal option for long-term environmental studies. Similarly, Moro *et al*. (2018) used an array of microalgae to design an optical bioassay for detecting marine pesticides. The primary obstacle in developing a microalgae biosensor in the marine environment is the high salinity of seawater. Therefore, the study evaluated microalgae from diverse groups and identified those that could adapt to this environment while simultaneously monitoring the chemical quality of the marine ecosystem.

The field of biosensors has recently made significant strides in detecting various pollutants in water, including heavy metals, pesticides, and organic compounds. As a result, they have emerged as a promising tool for environmental monitoring and management. It is crucial to prioritise the development of a microalgae-based biosensor that focuses on resource recycling, energy efficiency, and the promotion of circular biorefinery concepts. Moving forward, research should concentrate on optimising microalgae-based biosensors for commercial use and integrating them with advanced technologies such as nanotechnology (Allouzi *et al*., 2022). The combination of engineering, biotechnological, and computational techniques can improve the sensitivity, selectivity, and accuracy of microalgae biosensors.

Microalgae for Water Treatment in Constructed Wetland

Constructed Wetlands (CWs) are increasingly being recognised as an effective and sustainable approach for water treatment. Microalgae have a crucial role to play in the construction and operation of wetland systems, as shown in Figure 2. Within these systems, microalgae are highly beneficial for the removal of nutrients such as nitrogen and phosphorus from water, as they utilise these nutrients for their growth. According to Zhao *et al*. (2023), the inclusion of microalgae in the CWs led to a significant enhancement of approximately 20% in nitrogen removal. The tested microalgae strain (ZM-5) was found to be able to interact with denitrifying bacteria to compensate for the deficiency of the denitrifying stage in CWs. Yang *et al*. (2023) proposed a coupling process between algal ponds and constructed wetlands to enhance wastewater purification performance. The addition of 10.6 mg/(L·d) microalgae into the CWs resulted in a maximum total nitrogen removal of 90.3%, with 1 g of microalgae promoting an average of 0.291 g of nitrogen removal. Optimal parameters for the algal pond-CWs system included a 5-day Hydraulic Retention Time (HRT) for the algal pond and a 0.4% microalgae reuse rate. These parameters led to total nitrogen and total phosphorus removal rates of 94.18% and 85.80%, respectively.

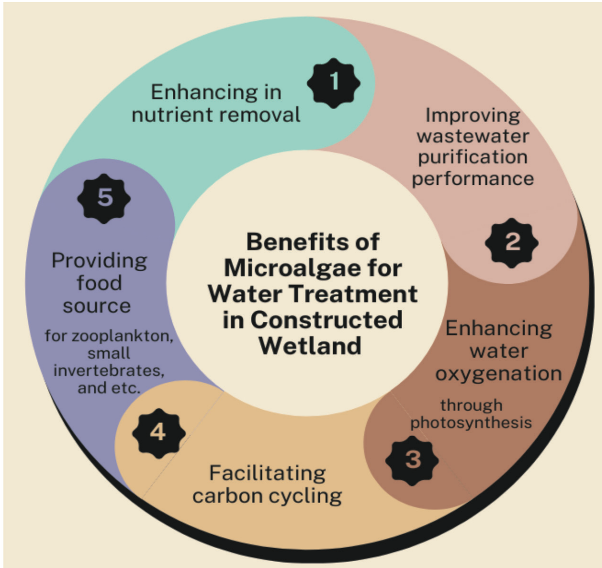

Figure 2: Roles of microalgae for a sustainable wastewater treatment approach in a constructed wetland

The incorporation of microalgae into wetland environments enhances water oxygenation through photosynthesis while also facilitating carbon cycling. Traditional methods of oxygen supplementation such as atmospheric oxygenation and plant root release are inadequate for the nitrification process. Microalgae, on the other hand, utilise atmospheric CO_2, release oxygen, and concurrently absorb nitrogen and phosphorus from water. This photosynthetic growth enables the conversion of pollutants into bio-resources (Zhuang *et al*., 2020). Furthermore, the treatment of wastewater in algal ponds results in increased oxygen content and C/N ratio. Li *et al*. (2022) conducted laboratory experiments to investigate the effects of nutrient load distribution and microalgae addition in constructed wetlands on pollutant removal. The algal pond demonstrated significant removal of NH4-N, with rates of 34.8% (HRT = 3 d) and 64.8% (HRT = 5 d) under favourable oxygenation conditions.

Microalgae provide a food source for various organisms such as zooplankton, small invertebrates, and other microorganisms in CWs ecosystems. Importantly, the formation of biofilms involves complex communities of microorganisms attaching to submerged surfaces. Microalgal cells form biofilms on solid substrates through the adhesion of cells and the production of an extracellular polymeric matrix, allowing for nutrient assimilation and photosynthesis near the surface of the biofilm (Nadell *et al*., 2015). The utilisation of microalgal biofilms has emerged as a promising alternative approach for cultivation.

Biofilms help to improve water quality by acting as a filter, trapping suspended particles and organic matter. Zhang *et al*. (2023) studied the microbial community in a continuous-flow microalgal-bacterial biofilm photoreactor for municipal wastewater treatment. The biofilms were successfully developed at a 9-hour Hydraulic Retention Time (HRT) without external aeration, achieving a biofilm concentration of approximately 4,690 mg/L in a steady state. The microalgal-bacterial biofilms achieved significant removal rates, with around 90% removal of total organic carbon, 71.4% removal of total nitrogen, and 72.6% removal of phosphorus through assimilation in the steady-state photoreactor run at a 12-hour HRT with external aeration.

Biomonitoring of urban wastewater treated by an integrated system combining microalgae and constructed wetlands has been reported by Silveira *et al*. (2020). A novel integrated system consisting of an anaerobic reactor, microalgae, and constructed wetlands was proposed for the detoxification of wastewater in the study, with a hydraulic detention time of 17 days. The results demonstrated that the integrated system achieved significant reductions in COD and BOD, as well as an impressive removal rate of nearly 98% for N-NH3, surpassing the performance of the tested conventional wastewater treatment plant.

Based on the aforementioned studies, optimal incorporation of microalgae into wetland construction necessitates careful consideration of factors such as species selection, environmental conditions, and nutrient availability. The design and management of the wetland should be tailored to enhance the growth and sustainable performance of microalgae populations. Leveraging the unique capabilities of microalgae, wetlands can achieve enhanced efficacy in wastewater treatment, water quality improvement, and support for essential ecological processes.

High Value-added Products from Microalgae Cultivation in Wastewater

Biofuels

Microalgae offer a promising source for biofuels due to their high lipid, carbohydrate, and protein content. Among these components, lipids have gained significant attention as they structurally resemble crude oil hydrocarbons and can be directly converted through transesterification for biodiesel production. In a study conducted by Chakravarty and Mallick (2019), a biphasic optimisation technique was employed to produce biodiesel from an aboriginal green microalga, *Selenastrum* sp. GA66. This technique aimed to reduce the loss of biomass and lipid due to stress, resulting in a 2.6-fold increase in lipid yield and 2.2-fold higher productivity.

Fatty acid profiling of the biodiesel samples revealed a higher degree of saturation and a lower level of unsaturation. Another study focused on the microalga *Micractinium* sp. IC-76, which was cultivated using a 110-L flat panel photobioreactor (Piligaev *et al*., 2018). The highest content of neutral lipids (44.1%) was observed on the 17th day of cultivation, comprising mainly C16:0, C16:2, and C18:2 fatty acids. Enzymatic transesterification was subsequently performed on the harvested lipids using cross-linked lipase aggregates as the biocatalyst, resulting in 92% biodiesel production.

The feasibility of microalgae as a viable and renewable energy source has been demonstrated in several countries, including Brazil, Japan, China, and the United States (Aratboni *et al*., 2019). The production of various type of biofuels such as biodiesel, bioethanol, bio-hydrogen, bio-oil, and biomethane, involves distinct production processes that are tailored to each specific type. These processes include transesterification for biodiesel (Chhandama *et al*., 2021), fermentation for bioethanol (Constantino *et al*., 2021), photobiological processes for bio-hydrogen (Li *et al*., 2021), anaerobic digestion for biomethane (Xiao *et al*., 2019), and either pyrolysis or liquefaction for bio-oil (Sekar *et al*., 2021). Understanding the different production methods for each biofuel type is crucial for effectively utilising these sustainable energy sources in the transition toward a cleaner, greener future. The advantages and disadvantages of the process are tabulated in Table 3.

Bio-oil from microalgae is produced via pyrolysis or liquefaction processes. Kaige *et al*. (2013) have demonstrated fast pyrolysis of microalgae remnants in a fluidised bed reactor for bio-oil and biochar production. The lipid for *C. vulgaris* biomass was first solvent-extracted while the remnants were used as feedstock for fast pyrolysis experiments using a fluidised bed reactor at 500°C. The pyrolysis treatment combined an oxygen-free thermochemical process in which the temperature ranged between 300°C and 700°C to yield the bio-oil, biochar, and gas at 53 wt.%, 31 wt.%, and

Table 3: The advantages and disadvantages of biodiesel, bioethanol, biomethane, bio-hydrogen, and bio-oil production processes

Biofuel Products	Processes	Advantages	Disadvantages	References
Biodiesel	Transesterification	Lower sulphur emissions	Conversion yield depends on the efficiency of the catalyst	(McCarthy *et al.*, 2011)
Bioethanol	Fermentation	Non-toxic and biodegradable	High production cost to grow the fermentation microorganism	(Phwan *et al.*, 2018)
Bioethanol	Enzymatic hydrolysis	Efficient bioethanol conversion using fermentable sugars	Slow efficiency of hydrolysis and expensive enzyme	(Lee *et al.*, 2019)
Bioethanol	Solar drying	The cost is inexpensive	Required long drying time, large area, and chances of losing biomass content	(Kandasamy *et al.*, 2022)
Bioethanol	Sprig drying	Extract high-value compounds	Costly and highly reduces microalgae's pigment	
Bioethanol	Free drying	Removes oil	Expensive and requires complicated vast operations	
Biomethane	Anaerobic digestion	The dewatering stage is unnecessary, hence, reducing energy consumption	Require anaerobic conditions	(Xiao *et al.*, 2019)
Bio-hydrogen	Bio-photolysis	Low environmental impact	Low-yield product	(Wiranarongkorn *et al.*, 2021)
Bio-oil	Pyrolysis/liquefaction	Produce other valuable products such as biochar, biogas	High energy requirement	(Sekar *et al.*, 2021)

10 wt.%, respectively (Sekar *et al*., 2021). Bio-oil and biochar production from algae through slow pyrolysis was explored by Chaiwong *et al*. (2013) on dried *Spirulina* sp. algae who observed a maximum degradation temperature of 322°C.

For methane production, pre-treatment of microalgae biomass becomes mandatory to improve the accessibility of organic matter. For instance, Xiao *et al*. (2019) used a solar-driven hydrothermal pre-treatment on microalgae biomass, which successfully produced a 57% improvement in biomethane production over the non-treated batch. According to Xiao *et al*. (2019), different microalgae strains may have a distinct biomethane potential, which is due not only to species differences in biomethane production but also to numerous factors such as process parameters, which have a significant impact on the overall yield and efficiency of the process. Microalgae have a higher congenital efficiency of solar energy conversion than terrestrial plants, which is approximately 3-8% higher.

Microalgae produce bio-hydrogen through a biological process in the presence of sunlight (bio-photolysis) or a fermentation process (dark or photo fermentation). Wiranarongkorn *et al*. (2021) reported that bio-hydrogen is better in conditions with fewer contaminants and high energy density. Nevertheless, the current low production yield limits the large-scale applications of microalgae-based biohydrogen, as described by Limongi *et al*. (2021). According to Li *et al*. (2021), several factors affect bio-hydrogen production from microalgae. For example, the energy conversion efficiency is dependent on a variety of operational parameters such as C:N ratio, temperature, pH, hydraulic retention time, and microbial community proliferation. These parameters affect the performance and efficiency of the dark and photo-fermentation processes.

Carotenoids

Tetraterpene pigments, commonly known as carotenoids, come in yellow, orange, red, and purple hues. Carotenoids are the most abundant pigments in nature, found in photosynthetic bacteria, algae, plants, animals, and certain fungi and archaea (Maoka, 2019). Carotenoids are a subtype of pigments, which also include chlorophylls, phycobilins, and xanthophylls (Sirohi *et al*., 2022).

Microalgae have gained increasing attention since they represent one of the most promising sources of bioactive compounds and functional ingredients within the pharmaceutical and food industries. Extracts from various microalgae such as *Haematococcus pluvialis*, *Nannochloropsis oceanica*, *Tisochrysis lutea*, and *Porphyridium cruentum* are enriched in carotenoids and have been tested for their neuroprotective properties through in vitro assays (Gallego *et al*., 2022). Simultaneous production of carotenoids and chemical building blocks precursors from Chlorophyta microalgae has been done by Rengel *et al*. (2022). The study evaluated the content of lipophilic coproducts in the microalga *Chlamydomonas reinhardtii* under different nutritional conditions. Under optimal conditions, the acidic hydrolysis of the algal biomass produced high-value-added glucose, levulinic acid, and 5′-hydroxymethylfurfural.

Meanwhile, Gui *et al*. (2022) investigated five different types of microalgae as aquaculture feed and their effects on improving the growth and health conditions of *Artemia nauplii*. The results showed that carotenoids from microalgae could efficiently transfer to *Artemia nauplii* and contribute to their improved growth and health conditions. In comparison with fatty acids, carotenoids had a greater impact on the survival rate, average body length, total effective body length, and total antioxidant capacity of *Artemia nauplii*.

Additionally, the immunity of *Artemia nauplii* was enhanced, as evidenced by their strong resistance against Vibrio parahaemolyticus-induced infection. Literature has reported a positive correlation between carotenoid consumption and serum low-density lipoprotein (HDL) content (Jeon *et al*., 2018). Nascimento *et al*. (2019) studied the effects of microalgae carotenoids from *Scenedesmus obliquus* on lipid peroxidation, the antioxidant defence system, and serum lipid profile in male mice. The results showed an increase in lipid peroxidation in the liver (160%) and serum triglycerides (60%) were observed using a higher dose of carotenoids.

Pigments

The market now has a strong demand for natural pigments derived from microalgae, which are widely used in a range of industrial applications, including food, nutraceuticals, pharmaceuticals, aquaculture, and cosmetics. Chlorophyll a, b, β-carotene, prasinoxanthin, siphonaxanthin, astaxanthin, fucoxanthin, and diadinoxanthin are among the types of microalgal pigments as summarised by Begum *et al*. (2016). These pigments serve as renewable, natural colour enhancers for food and feed products while also offering certain health benefits. Dagnino-Leone *et al*. (2022) have noted that phycobiliproteins exhibit fluorescence properties that can be advantageous for various industries, including pharmaceuticals, food, and cosmetics.

Phycobiliproteins have been sourced from numerous microalgae species, although their commercial production primarily relies on *Arthrospira* spp. (Spirulina) for phycocyanin and *Porphyridium* spp. for phycoerythrin (Bayu *et al*., 2022). The physicochemical factors for the two-stage cultivation of newly isolated oleaginous microalgae from a local lake have been optimised by Maneechote *et al*. (2021) as promising sources of pigments. Optimal conditions were obtained using the addition of 0.5% salt, the slightly high light intensity of 55 μmol·photon·m^{-2} s^{-1}, and a temperature of 30°C. Under nitrogen starvation, the selected strains showed a 1.3-1.6-fold increase in lipid content but with decreased pigment contents. *Scenedesmus* sp. SPP and *Chlorella* sp. PPS was observed producing high pigments while marine *Chlorella* sp. lipids showed excellent biodiesel properties.

Proteins

Some microalgae species are rich in protein, which can be used as a protein supplement in food and feed. Microalgae protein contains all the essential amino acids required by humans and can constitute up to 70% of its weight, making it an excellent source of protein. Spirulina is a typical high-protein microalga suitable as a food supplement. Colla *et al*. (2007) produced *Spirulina platensis* for nutraceuticals under different temperature and nitrogen regimes.

Meanwhile, Letras *et al*. (2022) used 3D printing technology to create an innovative gluten-free cereal snack that was nutritionally improved by incorporating *Spirulina platensis* and *Chlorella vulgaris*. Microalgae protein is increasingly being used as a sustainable alternative to traditional protein sources such as animal products. Lamminen *et al*. (2019) fed four cows with different protein supplements, including *Spirulina platensis*, *Chlorella vulgaris*, a mixture of *C. vulgaris* and *Nannochloropsis gaditana*, and soya bean meal.

The study found that the microalgae diets resulted in numerically higher energy-corrected milk yield and tended to increase milk fat and fatty acid concentrations compared to the soya bean meal. The study suggests that microalgae can be a comparable protein feed to soya bean meal in dairy cow nutrition, especially if the palatability of microalgae can be improved. Seelam *et al*. (2022) utilised nutrient recycling from digested waste to produce protein-rich microalgae for animal feed application. Their study explored the use of paper filtration as a pre-treatment technique to improve the physicochemical properties of food waste-based digestate for cultivating *Desmodesmus* sp. and *Chlorella vulgaris*. Phosphorus supplementation has improved the growth at higher digestate concentrations, indicating the importance of a balanced growth medium.

Antioxidants Compounds

Microalgae produce a wide range of antioxidants, including carotenoids, phycobilins, tocopherols, and phenolic compounds. These compounds can be extracted from microalgae and used as natural antioxidants in the food and cosmetic industries. The study by Gauthier *et al*. (2020) showed that microalgae produced significant antioxidant compounds under stress conditions. Different microalgal species responded differently to various stresses, with some showing an increase in enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase while others exhibited an increase in non-enzymatic antioxidants like carotenes, xanthophylls, and flavonoids.

According to López-Hernández *et al*. (2020), the production of bioactive compounds depends on three important factors: The microalga species used, the composition of the growth medium (including the amount and type of light provided), and the design and operational characteristics of the photobioreactor. Their study showed that microalgae cultivation in a continuous culture system significantly enhanced biomass productivity by 5.9 to 6.3 times compared to batch cultures. Phenols, terpenoids, and alkaloids were produced by *Spirulina platensis*, *Isochrysis galbana*, and *Tetraselmis suecica* while phycocyanin and allophycocyanin were only produced by *S. platensis* and *Porphyridium cruentum*.

On the other hand, the production of various antioxidant compounds in microalgae is influenced by light intensity. In a study by Coulombier *et al*. (2020), it was observed that high light intensity increased peroxyl radical scavenging capacity, whereas low light intensity led to increased inhibition of lipid peroxidation for most of the species tested. Similarly, the study by Cheirsilp *et al*. (2022) using a two-stage LED light illumination strategy demonstrated increased astaxanthin production about 1.22- to 2.07-fold higher than that achieved through one-stage cultivation.

Nevertheless, microalgae cultivated in wastewater are not recommended for human consumption due to potential contamination with toxic bacteria, pathogens, and biotoxins. While commonly used for biofuels, bioplastics, and biofertilisers, they may be suitable for animal feed if rigorously processed to ensure safety (Su *et al*., 2023). Species like Chlamydomonas acidophila, which absorb heavy metals, are particularly unsuitable for human use (Díaz *et al*., 2020). For pharmaceuticals or cosmetics, strict pre-treatment of wastewater is essential to eliminate contaminants and ensure product safety (Jasni *et al*., 2021).

Challenges in Microalgae Cultivation in Wastewater

Microalgae Selection

The selection of a suitable microalgae cultivation system ensures high phytoremediation efficiency and biofuel yield. This all depends on the microalgae category to be cultivated, namely, autotrophic, heterotrophic, mixotrophic, and photoheterotrophic (Chen & Durbin, 1994). Nonetheless, mixotrophic microalgae are the best option for maximising biomass and lipid productivity (Scarsella *et al*., 2010). Mixotrophic microalgae are adept at using inorganic and organic carbon sources for growth in the presence of light, where photoautotrophic and heterotrophic processes can simultaneously occur (Caporgno *et al*., 2019). Studies have identified *C. vulgaris* as an ideal candidate for biodiesel production due to its high biomass yield and lipid content.

Under mixotrophic conditions, *Chlorella* sp. had a maximum lipid productivity of 3.5-fold higher than the control medium with glycerol supplementation (Sharma *et al*., 2016). Álvarez-Díaz *et al*. (2017) cultured seven species of microalgae in both wastewater and synthetic media and found that *Chlorella vulgaris*, *C. kessleri*, and *Scenedesmus obliquus* produced higher biomass productivity in wastewater than in synthetic medium. In the study by Kabir *et al*. (2020), microalgae *C. vulgaris* yielded the highest biomass in BG-11 medium (1.86 ± 0.1 g/L) (1.14 ± 0.3 g/L), followed by *S. obliquus* and had the highest lipid content of 35.1 ± 1.0% under heterotrophic cultivation conditions. Various microalgae strains have been found to exhibit over 75% efficiency in Chemical Oxygen Demand (COD) removal when grown in wastewater, with different strains exhibiting different COD removal efficiency while producing desirable lipid products (Table 4).

Growth Condition

While microalgae cultivation in wastewater is relatively straightforward because of its simple growth requirements, several environmental factors influence optimum algal growth and product accumulation. Such factors include the availability and concentration of micronutrients and macronutrients, carbon dioxide, temperature, pH, light intensity, and photoperiods (Saad *et al*., 2019). It is pertinent that the conditions are appropriately addressed to maximise biofuel production to offset the high processing costs. The quantity and quality of light determine the amount of vital energy for the microalgal photosynthetic process. The balance between dark and light regimes greatly influences algal growth and biomass production, as heightened frequencies of the light/dark cycles might substantially enhance microalgae's productivity and photosynthetic efficiency (Grobbelaar, 2009).

Chin *et al*. (2023) investigated the effects of light intensity and nitrogen concentration to enhance lipid production of four tropical microalgae: *Ankistrodesmus falcatus*, *Chlorella emersonii*, *Chaetoceros muelleri*, and *Isochrysis galbana*. Under optimised conditions, *C. muelleri* and *I. galbana* were found to produce the highest lipid content, up to 43.40% dry weight, and desirable fatty acid profiles characterised by a high concentration of saturated fatty acids (43.89%-48.00%) and monounsaturated fatty acids (35.30%-38.44%).

Meanwhile, Luo *et al*. (2019) reported that pH media substantially impact the cell surface features, which can alter microalgae's biochemical metabolism and enzyme system activity. The cellular enzymatic reaction of microalgae is also greatly impacted by temperature, which modulates metabolic reactions, transport rate within the cell, and cell regulation, ultimately affects composition and structure. High temperatures can cause protein breakdown and lead to cell death, as reported by Serra-Maia *et al*. (2016).

Literature has shown that CO_2 aeration can improve the cultivation of microalgae in media derived from wastewater. Han *et al*. (2016) reported that the highest microalgal lipid productivity was achieved at an aeration rate of 0.2 vvm in wastewater cultivation. The optimised aeration rate also improved the fatty acid profile, particularly the production of monounsaturated fatty acids, mainly C18:1, which was about 1.8 times higher than that of the non-aerated *S. obliquus*. Furthermore, aeration with 15% CO_2 significantly enhanced lipid accumulation, resulting in a total lipid content of 25.6%, which was twice the yield observed at 5% CO_2 under the specific conditions studied by Mohsenpour and Willoughby (2015).

On the other hand, Wu *et al*. (2017) showed that wastewater concentration is a factor that warrants our attention as it could alter waste removal efficiency and product formation. They found that when cultivating microalgae strains in textile wastewater at dilution rates ranging from 0% to 80%, a dilution rate of 10% was optimal for removing NH_4+-N after seven days. Pereira *et al*. (2020) also investigated the effect of dilution rate (0.1 to 0.5 day^{-1}) on productivity and waste removal using secondary effluent as a culture medium for *Chlorella* sp. L06. They observed that lower dilution rates led to higher biomass lipid content and energy storage while higher dilution rates improved waste removal efficiency.

Under limiting culture conditions, microalgae's biosynthetic pathway is altered to favour larger quantities of neutral lipids (20%-

Table 4: Different types of microalgae strains are categorised based on their performances in different types of wastewater-based media

Representative Species	Type of Wastewaters	Total Lipid Content % (W/W)	Total Lipid Productivity (Mg/L/D)	Growth Rate (*μ*max)	COD Removal Efficiency (%)	References
Chlorella vulgaris	-	48 ± 0	-	-	-	(Thirugnanasambantham *et al*., 2020)
	Municipal wastewater	39 ± 2	163	1.65	100 ± 0	(Ma *et al*., 2016)
Desmodesmus sp. ASK01	Raw dairy effluent	22.70	23.21	0.23	87.85	(Pandey *et al*., 2019)
Chlorella sp. ASK14	Raw dairy effluent	13.80	12.43	0.31	92.92	
Chlorella sp. ASK25	Raw dairy effluent	21.20	26.22	0.30	84.92	
Chlorella sp. ASK27	Raw dairy effluent	25.80	27.63	0.26	91.90	
Scenedesmus sp. ASK16	Raw dairy effluent	24.60	16.03	0.26	87.85	
Scenedesmus sp. ASK22	Raw dairy effluent	30.70	31.16	0.29	90.50	
Nannochloropsis oculata	Palm oil mill effluent	52.00	-	-	71.00	(Emparan *et al*., 2020)
Chlorella thermophila MF179624	Campus sewage wastewater	21.50	81.38	-	82.53 ± 4.37	(Gebremedhin *et al*., 2018)
Chlorella vulgaris SAG 211-19	Seafood wastewater effluent	32.15 ± 1.45	-	0.65 ± 0.01	-	(Nguyen *et al*., 2019)
Chlorella sp.	Textile wastewater	16.60	9.60	-	75 ± 3	(Wu *et al*., 2017)
	Municipal wastewater	-	-	0.24 ± 0.01	91.6 ± 1.4	(Pereira *et al*., 2020)
Chlorococcum sp.	Municipal wastewater	-	-	0.18 ± 0.01	80.1 ± 6.0	
Scenedesmus sp.	Municipal wastewater	-	-	0.19 ± 0.01	80.9 ± 2.2	
Tetradesmus sp.	Municipal wastewater	-	-	0.20 ± 0.01	87.7 ± 1.0	
Chlorella sorokiniana	Municipal wastewater and piggery wastewater	-	-	0.28	92.2	(Leite *et al*., 2019)
Scenedesmus obliquus	Raw cattle wastewater	22.7 ± 1.8	> 10.5	-	-	(Xia *et al*., 2020)
Chlorella sorokiniana CY-1	Palm oil mill effluent	20.90	-	-	45.05	(Cheah *et al*., 2018)
Chlorella vulgaris ESP-31	Palm oil mill effluent	17.50	-	-	-	

50% of dry weight), namely triacylglycerols, primarily deposited in lipid bodies in the cytosol. Stress conditions, such as extreme pH and limited nutrients ultimately maximise lipid production in certain microalgal species, producing the highest yields of biofuels. There are reported improvements in lipid production by as much as 70% under stress conditions, as reported by Katiyar *et al*. (2017).

Conversely, a nutrient-saturated condition sees the light as a critical factor for microalgae photosynthetic activity. A specific saturating light level leads to a maximum growth rate, whereas a light intensity far above the saturation level inhibits microalgal growth. Whereas light intensity far below the saturating light level becomes growth-limiting.

In addition, the highest lipid yield of microalgae cultivated in wastewater varies based on several factors, including microalgae species, the environment of the medium (dilution level of the wastewater), co-cultivation or single cultivation, nutrient composition, metal ions, and lighting conditions. Thus, the co-cultivation of microalgae (*Ankistrodesmus fusiformis*) with a dilution level of 59.88% of wastewater (palm oil mill effluent) was the most optimum for achieving 0.012 mg/L/d of lipid productivity (Justine *et al*., 2023).

Furthermore, according to Han *et al*. (2021), *Chlorella pyrenoidosa* yielded a maximum lipid of 0.18 g/L at a total phosphorus concentration of 5 mg/L and light intensity of 4000 lux with 9:15 h L/D. However, when treated with metal ion, Mg^{2+} concentration (0.5 mM), the total lipid yield reached the maximum (0.25 g/L). In addition, they stated that the most optimum culture conditions were as follows: Photoperiodicity of 23:1 h L/D; ammonia nitrogen with 125 mg/L; Fe^{3+}, 0 mM; light intensity, 9,000 lux; phosphorus, 14 mg/L; Mg^{2+}, 0.18 mM; and CO_2, 1.3 vol%. Moreover, the maximum theoretical lipid yield was 0.31 g/L.

Upstream Cultivation

Microalgae cultivation requires a reactor vessel, with two main type of systems: Closed photobioreactors (PBRs) and open pond systems. Ramirez (2015) recommended closed systems for better control of CO_2 and light. PBRs reduce contamination risks and offer higher production yields, as demonstrated by Ananthi *et al*. (2021). These systems utilise natural sunlight and artificial lighting (e.g., LEDs and fluorescent tubes) to provide even light distribution, promoting biomass production (Khan *et al*., 2018).

However, variations in light intensity and CO_2 levels can affect microalgal growth. While PBRs can convert 9-%10% of solar energy into biomass (77 g/biomass/m²/day or 280 tons/ha/year), they tend to yield lower biofuels due to reduced radiation. Other limitations include poor mixing, CO_2 loss, oxygen accumulation, limited scalability, and high capital and operational costs due to the need for artificial light and maintenance as reported by Pruvost *et al*. (2016).

In contrast, open pond systems are simpler and more cost-effective to construct than PBRs. These systems can be natural (lakes, lagoons) or artificial ponds and they align more closely with the natural environment of microalgae (Tan *et al*., 2020). Open systems, however, offer lower yields due to low cell density, which limits biofuel production according to Rafa *et al*. (2021). They also face challenges such as high water demands, high evaporation rates, and temperature fluctuations, which can reduce productivity outside the 20°C-35°C range. Moreover, open ponds are more prone to contamination by unwanted microorganisms, which can outcompete the target species, an issue less common in PBRs (Chahal *et al*., 2016). The capital and operational costs of open ponds can be high if using concrete or plastic linings to prevent sedimentation (Das & Obbard, 2010).

Xiaogang *et al*. (2020) demonstrated that two primary systems are used for microalgae cultivation in wastewater: closed Photobioreactors (PBRs) and Open Pond Systems (OPs). Their discussion indicates that various open pond systems cultivated with wastewater support a range of microalgae species with different lipid production, biomass productivity, and nutrient removal efficiencies.

Among these, the raceway pond system is noted for its economic viability due to its low power requirements and ease of maintenance. In contrast, a previous study by Umamaheswari and Shanthakumar (2019) found that PBRs had the highest biomass productivity, achieving 340 ± 2 mg/L/d, with ammoniacal nitrogen (NH_3-N) removal of 96.12 ± 0.21% and phosphate (PO_4-P) removal of 97.58 ± 0.18%. Additionally, the PBR system yielded the highest lipid (12% of biomass), protein (40%), and carbohydrate (20%) content, outperforming raceway ponds in terms of nutrient removal and lipid production.

However, using wastewater in PBRs for microalgae cultivation presents specific challenges. A major issue is the supply of carbon dioxide, which is essential for optimal biomass yield and often requires external supplementation (Magalhães *et al*., 2022). Additionally, PBRs typically incur higher cultivation costs compared to open systems, particularly due to the infrastructure and energy requirements (Peter *et al*., 2022). Another limitation is the risk of contamination when using wastewater in PBRs, as the medium can introduce harmful microorganisms, which may affect the health and productivity of the microalgae culture (Khor *et al*., 2022). These drawbacks highlight the need for further optimisation of PBRs when using wastewater as a cultivation medium.

Harvesting Process

Kumar *et al*. (2015) have comprehensively reviewed various lipid extraction methods from microalgae via solvent extraction procedures, mechanical approaches, and solvent-free procedures, in addition to the latest extraction technologies. However, the microalgae harvesting process poses a formidable challenge in scaling up the technology. Harvesting and dewatering are the major downstream processes necessary before the microalgal products are usable for different applications. The diversity in the microalgal chemical composition often requires a fractionation step to maximise the desired molecule's yield and minimise unwanted and inhibitory products (Nitsos *et al*., 2020). For these reasons, the harvesting process of microalgae-produced lipids accounts for up to 20% to 30% of the total production cost. This is attributable to the microalgae's low density in water and minute size (typically ~1 g/L) (Singh & Patidar, 2018).

Sedimentation velocity among microalgae species can differ by several orders of magnitude based on density, cell size, and shape. According to Nitsos *et al*. (2020), small-diameter and low-density microalgae sediment are considerably slower than larger and denser cells. So far, gravity-assisted sedimentation is applicable for mixed microalgae cultures containing colonies dominated by species with large settling velocities. Manheim and Nelson (2013) discovered a major issue when slow-settling microalgae are the dominant colony in the flocculation of two algae species, hence, diminishing the harvesting efficiency despite prolonged settling periods for up to 24 hours. Although economically feasible harvesting techniques are in place through successive flocculation and sedimentation processes, the method requires a longer settling time, which delays the harvesting process (Loo *et al*., 2021). It is because gravity sedimentation treatment alone is insufficient to induce biomass dewatering. Reduced harvesting cost and energy by a factor of 30 is achievable when integrated with other physical treatments, i.e., centrifugation (Nitsos *et al*., 2020).

Hydrothermal technologies involving pyrolysis, gasification, and hydrothermal treatment were introduced for expedient dewatering and bioenergy conversion of microalgal biomass. Hydrothermal processes, which categorically include carbonisation, liquefaction, and gasification, involve the thermal disintegration of wet microalgae biomass from pre-treatment by gravity-assisted dewatering. These processes bring about varying compositions of the biofuel end products.

However, these treatments to produce viscous bio-crude oil are energy-intensive and incur costs. For instance, the high treatment temperatures for liquefaction are executed at 250°C-450°C at 40-165 bar (Eboibi *et al*., 2014). The pyrolysis treatment occurs at temperatures between 400°C and 500°C (for 2-3 s) to obtain liquid bio-crude oil containing tars, heavier hydrocarbons, and water. The greener approach by gasification for harvesting bio-crude oil using supercritical water remains prohibitive, too. The system demands high-energy and special equipment to sustain the high temperature and pressure, typically between 220°C-380°C, for further transesterification (Salaheldeen *et al*., 2021). In any case, much work is necessary for improving hydrothermal technology for harvesting microalgal lipids, which warrants interesting future studies.

Extraction Process

Microalgae have a very thick and rigid cell wall, causing certain extraction processes to yield less than 50% of the desired product per biomass dry weight. Aside from the thick cell wall, the bioproducts are confined within the cytoplasm and bonded to the cell membrane's phospholipid bilayer. This further adds to the complexities of their extraction. This is among the reasons for the failure of solvents to penetrate through the cell wall, leading to poor contact between the extracting solvent and lipids, as Quan *et al*. (2021) did in their study. The authors reported effective lipid extraction methods from underwatered microalgae liquid using subcritical dimethyl ether as a green solvent. The addition of chemical flocculants, such as $AlCl_3$ and $FeCl_3$ expedites the sedimentation process. Nevertheless, efficiency comes at a high cost to the environment.

To mitigate environmental damage, less toxic and more cost-effective solvents and flocculants could be better choices. The irresponsible handling and/or disposal of these solvents may pose serious hazards to humans and the ecology (Mahmood *et al*., 2017). Hence, greener solvents with better environmental, health, and safety profiles are needed in creating sustainable biofuel production to meet the demands of the Sustainable Development Goal. Sicaire *et al*. (2015) proposed that new and greener classes of bio-based and deep eutectic solvents be concocted to substitute the hazardous petroleum-derived solvents. They experimented on rapeseed oil extraction at laboratory and pilot plant scales to determine lipid yields, extraction kinetics, and lipid composition.

Developing an effective cell disruption method is key to achieving the highest lipid yield for biofuel production. For example, Román *et al*. (2012) proposed pulverising the biomass with mortar and a pestle, using liquid nitrogen as a lyophilisation agent. The abrasive ice crystals formed inside the cells help liberate internal lipids to contact the extracting solvent. Halim *et al*. (2011) employed a reusable bead milling method, in which the microalgae were physically ground by agitation against bead mills. However, the mechanical means to break down the cell wall might not be cost-effective for mass production. Table 5 presents past research detailing lipid extraction from various microalgae biomasses and their corresponding yields.

Conclusions and Future Outlook

Microalgal technology is beneficial for combating both energy crises and environmental pollution. Microalgae cultivation can effectively absorb CO_2 in the environment via the photosynthesis pathway and produce valuable products as feedstock for biodiesel production, which is beneficial as a cost-effective and green alternative to depleting fossil-based fuel. Simultaneously, microalgae with a short life span and high growth rate can utilise nutrients present in wastewater, reducing the negative impact of industrial and agricultural wastes on the water stream. However, several critical

Table 5: Biofuel yield by various solvents and microalgae species

Microalgae	Solvents	Yield (% wt)	References
Botrycoccus sp.	Bligh and Dyer	28.1	(Lee *et al*., 2010)
Chlorococcum sp.	Supercritical CO_2	7.1	(Halim *et al*., 2011)
Chlorella vulgaris	Bligh Dyer and Hexane	0.88	(Tanzi *et al*., 2012)
Chlorella vulgaris	Bligh Dyer and Hexane	52.5	(Araujo *et al*., 2013)
Picochlorum sp.	Bligh Dyer and Ethanol	33.04	(Wang *et al*., 2021)
Chlorella vulgaris	Hexane	16.28	(Mahmood *et al*., 2017)
Nannochloropsis oculata	Ethanol	5.73	
Botryococcus braunili	-	25.0-75.0	(Culaba *et al*., 2020)
Crypthecodinium cohnii	-	20.0	
Cylindrotheca sp.	-	16.0-37.0	
Dunaliella primolecta	-	25.0-33.0	
Isochrysis sp.	-	> 20.0	

challenges for biofuel commercialisation are associated with upstream and downstream processing. Therefore, a wise selection of innovative technologies could ensure the long-term viability of biofuel production through microalgae cultivation.

The perspectives of microalgal technology demonstrate a fascinating future, although commercialisation of this technology is still in its infant stage. Several factors must be considered to propel the technology onto the global stage on a trajectory that displaces fossil fuels and limits global temperature increases. As cost reduction is a significant barrier to large-scale production, minimising the substrate cost for microalgae cultivation has been a major concern. Using agricultural waste as a low-cost medium could effectively reduce cultivation costs while providing essential nutrients (i.e., nitrogen, carbon, phosphorus, and other organic matter) for microalgae growth. The operating conditions for the production process must also be optimised for developing an economically and industrially feasible microalgal biofuel. Therefore, future research should focus on the optimal handling of microalgal residual materials after lipid recovery as feedstocks for bioethanol production or animal feed. Furthermore, the lipid-extracted biomass can be converted into novel biomaterials such as nanoparticles and carbon dots, ensuring complete utilisation of microalgae biomass in the biorefinery system.

The future direction for microalgal components such as astaxanthin, β-carotene, fucoxanthin, DHA, and EPA is highly promising, driven by their diverse applications in food, cosmetics, and pharmaceuticals. As demand grows for natural, plant-based ingredients, these bioactive compounds are expected to see expanded use in health supplements, functional foods, and skincare products. Additionally, emerging research on the antiviral properties of *Porphyridium* sp. and the potential for algae grown in wastewater to contribute to sustainable food systems offers exciting opportunities. Technological advancements in food safety such as IoT monitoring will be key to ensuring the safe integration of microalgae into mainstream markets.

Scholars and scientists have extensively investigated and reported on research and development in various stages of microalgal-based biofuel production up to proof-of-concept. However, given the wealth of data gathered from numerous comprehensive studies, such bench-scale technologies should advance to commercial practice. It takes interdisciplinary collaboration between various parties, particularly scientists, relevant industries, and government agencies to translate such knowledge into tangible technology. The funding of research and development activities by the federal and state governments might also expedite the pace of technological advancement. This should be done in conjunction with government and official efforts to increase consumer awareness of the advantages of organic fuels.

Microalgal biofuel could be effectively commercialised sooner rather than later with consistent efforts and integrated technologies from cultivation to biodiesel production and the biorefinery concept. Hence, using a microalgae-assisted treatment on waste effluents could serve as the basis for simultaneous green waste management and economically sustainable bioenergy production.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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