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Review

Efficiency of Microalgae Employment in Nutrient Removal (Nitrogen and Phosphorous) from Municipal Wastewater

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Abstract: Growing population, industrialisation, and demand for resources put pressure on the delicate balance of the planet's ecosystems. From alternative sources of energy, healthier foods, cleaner water, and an overall more sustainable economy, the integration of microalgae in various industries, that otherwise are based on practices that hurt the environment, could be a successful solution. To reach that goal, further research is required on the complex relationship between microalgae and growth parameters (temperature, light intensity and spectrum, nutrient distribution, inhibiting factors, and so on). The scientific community successfully used microalgae to produce healthier foods, pigments, biofuel, animal fodder, methods for sequestering heavy metals, toxic compounds from water, and much more. In this review article, we approach the use of microalgae in municipal wastewater treatment, mainly for using nitrogen and phosphorous present in water as nutrients. Data were collected from articles published in the last 7 years (2018–2024). The results show that microalgae are very efficient at using N and P compounds from wastewater, as well as carbon, converting them in high-value substances (proteins, lipids, carbohydrates, etc.) with further applications in multiple industries.

Keywords: microalgae; wastewater; photobioreactor; nutrient removal; sustainability; resource recovery

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1. Introduction

Microalgae are microscopic organisms found in most ecosystems, with a large adaptability in freshwater, marine, and soil environments. With a high efficiency of converting solar radiation into valuable products, microalgae are a source of cellulose, carbohydrates, sugars, lipids, proteins, and bioactive compounds. Carbohydrates, lipids, and proteins stored in algae can be used for biofuel, biomethane, biodiesel, bioethanol, and biohydrogen production [1].

During the photosynthetic growth, microalgae will release oxygen into the atmosphere, sequester organic and inorganic carbon, bioaccumulate nutrients (nitrogen and phosphorous), and take in many pollutants from wastewater. Thus, microalgae could be successfully integrated in effluent treatment plants with multiple applications for microalgae-based

products. Microalgae growth in wastewater depends on several critical culture parameters such as CO_2 content, light intensity and photoperiod, initial inoculum level, nutrient concentration in the medium [2].

Freshwater is used for fulfilling multiple needs in our current society, from industrial to domestic uses. This generates vast quantities of wastewater, which pose a serious threat to ecosystems when discharged untreated into the environment [3]. The goal of municipal wastewater treatment with microalgae in photobioreactors is not only remediation of wastewater but also to enhanced biomass productivity for application in biofuel and feedstock, as wastewater acts as an inexpensive medium for microalgae biomass production [4].

According to the available data, approximatively 380 trillion litres of wastewater are produced globally, every year [5].

In a report published by the United Nations, the volume of treated wastewater varies greatly, from 70% in developed and developing countries to 38% in middle-income countries and 8% in low-income countries. Estimates show that 40% of the world's population will be affected by a freshwater shortage by 2030, with grave implications for our social and economic stability [6].

Municipal wastewater (MWW) is defined as wastewater discharged from houses, kitchens, bathrooms, laundry rooms, etc. The volume of MWW generated depends on the degree of urbanization and the population increase in an area. MWW contains lower levels of N (15–90 mg/L) and P (5–20 mg/L) compared to industrial or agricultural wastewater [7]. Multiple review articles focus on the use of microalgae for industrial or agricultural wastewater treatment, with less emphasis on municipal/urban/domestic wastewater treatment.

The most important nutrients in municipal wastewater are nitrogen (N) and phosphorous (P). The N and P concentrations found in MWW, from articles selected for this review, are between 19.5 and 576 mg/L for nitrogen and 0.6 and 116 mg/L for phosphorous [8–11]. The accepted quantities in drinking water should not exceed the limits established by WHO: 50 mg/L for nitrate and 3 mg/L for nitrite [12].

Municipal wastewater also contains different hazardous compounds, such as pesticides, polymers, microplastic, heavy metals, and pharmaceuticals. These chemical compounds at high concentrations have hazardous effects on humans and aquatic ecosystems via the food chain. According to the WHO's Guidelines For Drinking-Water Quality 2022, heavy metals should not exceed well-established limits in drinking water, as follows: 0.01 mg/L for arsenic, 0.003 mg/L for cadmium, 0.05 mg/L for chromium, 2 mg/L for copper, 0.01 mg/L for lead, 0.08 mg/L for manganese, 0.006 mg/L for mercury, 0.07 mg/L for nickel, and 0.04 mg/L for selenium [12].

Usually, MWW treatment requires physical, chemical, and biological methods.

The physical methods are represented by processes such as screening, sedimentation, aeration, filtration, and skimming in order to remove solid particles. Sedimentation represents the main physical technique for MWW treatment, in which the insoluble or heavy particles are suspended from the wastewater column and settled down at the bottom, and clean water is separated. Another method could be successfully employed by using specific filters (sand, clay beads, charcoal, etc.) used to pass the MWW in order to separate the contaminants and insoluble particles (grease, plastics, vegetal residues, etc.) [13].

The chemical methods are the most widely used MWW treatment techniques. Oxidizing chemicals (chlorine, ozone, etc.) are added to MWW to remove the organic content, to kill bacteria, and to reduce contaminants. Other chemical substances can be used to treat MWW, such as: coagulants, acids, or bases to neutralize the water and bring the pH to 7.

The biological methods employ microorganisms to break down the organic matter present in MWW (food residues, oils, human waste). Depending on the nature of degradation, biological treatments can be divided as follows:

- Aerobic decomposition, in which bacteria convert the organic matter into carbon dioxide, with the introduction on exogenous oxygen.
- Anaerobic decomposition, in which the waste is fermented at a specific temperature, in an oxygen-free process.
- Composting, in which MWW is treated with a source of carbon and atmospheric air.

The most used biological method for MWW treatment is the activated sludge. This represents a complex separation process between the solid and liquid phases of the MWW. This separation is considered successful when the minimum possible moisture is present in the solid phase and the minimum solid residues are present in the liquid phase. Thus, the remaining moisture in the sludge affects the disposal costs, and the processed water is further treated to remove the pollution load.

With a lower environmental impact compared to traditional forms of agriculture, microalgae are effective at removing pollutants and heavy metals from MWW, therefore having the potential to contribute to wastewater treatment and bioremediation [14]. Further research is required for developing microalgae strains that could improve the scalability with lower environmental requirements and higher production outputs [15]. However, any new species used for the production of nutraceuticals must comply with the EU's Novel Food regulations.

The review article from Muñoz and Guieysse, 2006, has put the basis for the use of microalgae and photooxygenation for the removal of pollutants during wastewater treatment [16]. In addition, different conditions and bioreactor configurations were applied, with the promising use of microalgae to produce high-value chemicals, biogas, or as feedstock.

As promising as microalgae are for the betterment of our future, there are still multiple knowledge gaps in microalgae production. There is a lack of knowledge regarding the complex interactions between microalgae and substances present in MWW and concerning the challenges associated with biomass separation. Further scientific progress is required in the methods for extracting valuable compounds (e.g., lipids and pigments), microalgae strain selection, microalgae–bacteria interactions, heavy metal concentrations, and others [17].

1.1. Microalgae Production

A significant number of studies have been carried out on the distribution of species of freshwater algal communities in diverse water resources. The most commonly cultivated microalgae species in Europe are *Chlorella* sp., *Nannochloropsis* sp., and *Haematococcus pluvialis*. Internationally, *Chlorella* and *Spirulina* are the two most cultivated species [18,19].

In terms of the scientific use of microalgae species, from the selected papers that approach the treatment of municipal wastewater using microalgae species (including blue-green bacteria or diatoms), the most researched species is *Chlorella vulgaris*. In Figure 1, the number of scientific papers for each microalgae species is presented.

In Europe, there are 89 companies producing microalgae. Most of them are located in France (over 60%). Microalgae (including the cyanobacteria Spirulina) production methods are land-based, most of them involving photobioreactors (71%), followed by ponds (19%) and fermenters (10%). The majority of Spirulina production (83%) takes place in ponds.

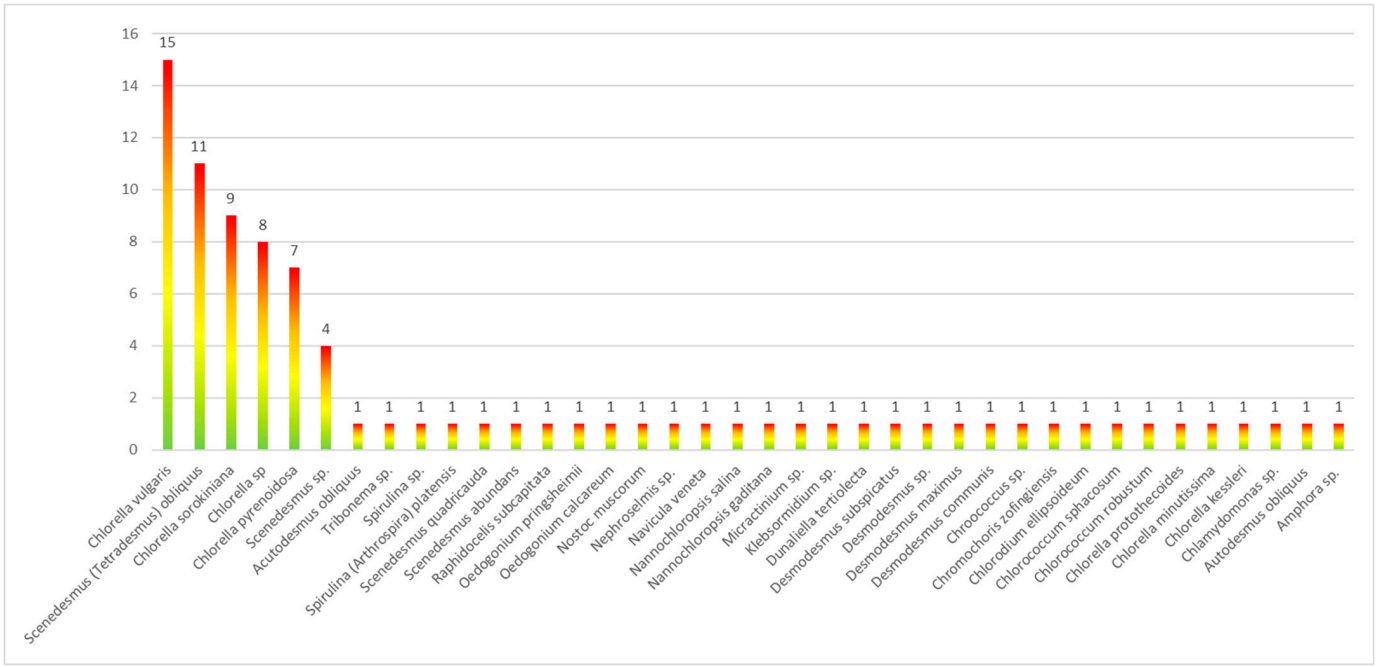

Figure 1. Distribution of microalgae species in the scientific papers selected in this review.

Microalgae production worldwide accounts for less than 1% of the total algae production (56,456 tons). Chinese production accounts for 97% of global yield for microalgae and cyanobacteria together, with higher *Spirulina* and *Arthrospira* production compared to other microalgae.

Microalgae biomass, in Europe, is primarily used for nutraceuticals (24%), cosmetics (24%), and for feed (19%). There is the potential use of microalgae for biofuels production, pharmaceuticals, bioplastics, and other bioproducts, but currently, the yield in these areas is still low, due to high costs of production and the need for further technological advancements [20].

1.2. Article Search and Selection

The involvement of microalgae in wastewater treatment is a topic that gains increasing interest as time goes by. As a positive consequence, a large number of scientific articles are generated. Since the applications of microalgae in wastewater treatment are vast, it was necessary to narrow down the selection of articles, choosing only those publications that were closest in topic to the one of interest in this review, the treatment of municipal wastewater using microalgae.

The searching and selection process was achieved by searching multiple journals, with specific keywords, at the beginning of the review. Only the significant key journals were chosen, based on a thorough analysis of previous articles that approached the same topic. The selected articles have a publish period from 2019 to 2023, covering the latest discoveries and improvements in MWW treatment in PBRs with microalgae, revealing a growing interest in the study of microalgae for municipal wastewater treatment during the recent years. Figure 2 represents the number of articles published each year that were selected for this review article.

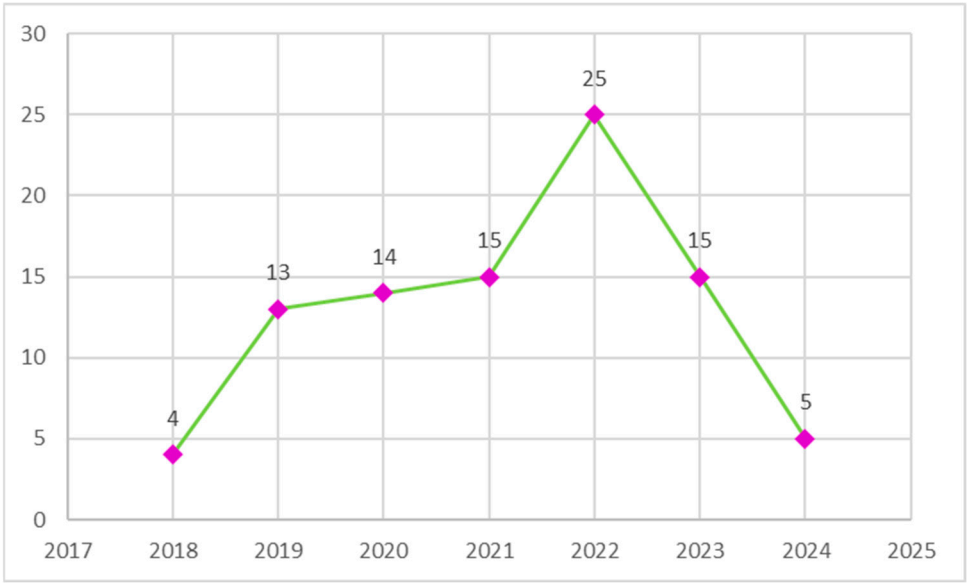

Figure 2. Scientific papers that explored the used of microalgae for municipal wastewater, published by year.

After the article-searching process, over 300 articles were retrieved, requiring further filtration and selection. Papers that had a clear presentation of the nutrient removal capacity of microalgae were selected, as well as those papers in which municipal or urban wastewater was used as a growing medium for microalgae. Figure 3 represents the number of articles in which each nutrient removal was analysed and where lipids or proteins were measured, showing an extensive focus on nitrogen compounds removal and lipid production.

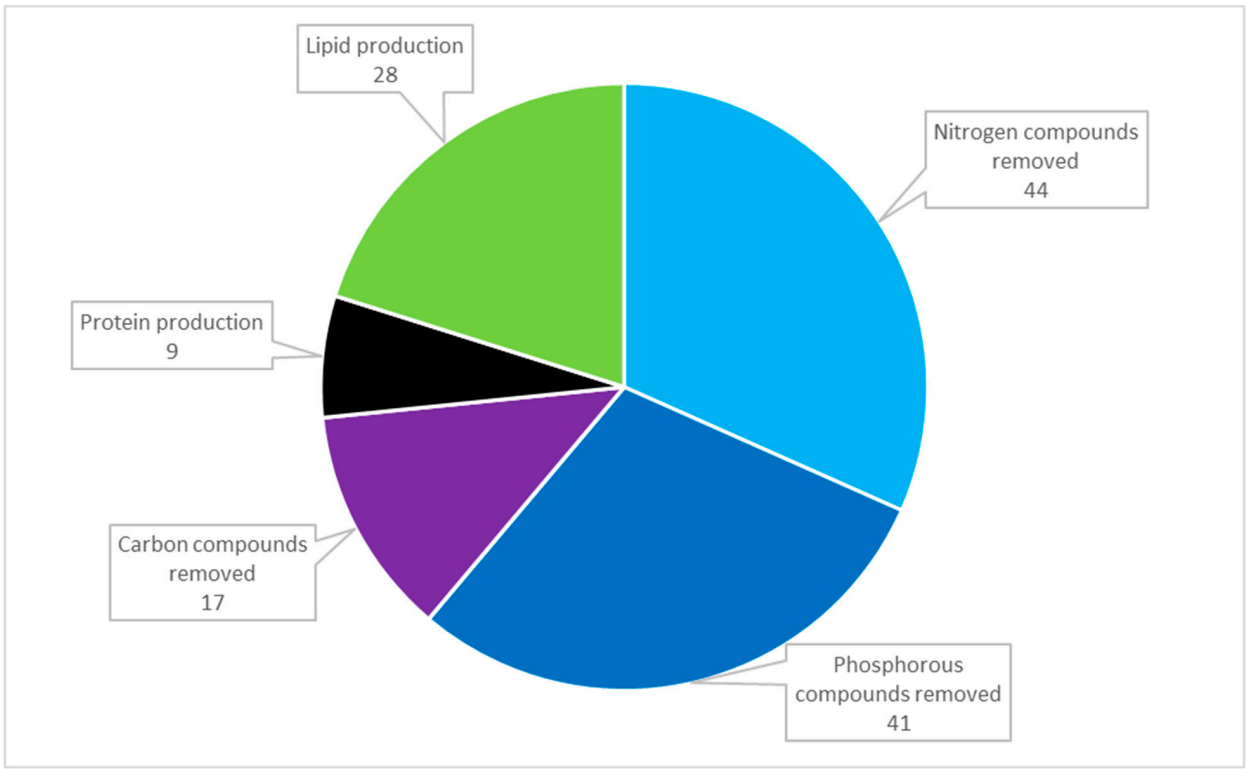

Figure 3. Number of scientific papers by type of nutrient removed and macronutrient produced, selected for the current review article.

The bibliographic resources that generated the most papers selected in this review were Science Direct and Google Scholar.

2. Microalgal Use in Wastewater Treatment

2.1. Production Methos

There are several methods considered for nutrient removal. This variety of mechanisms for the removal of phosphorus and nitrogen in PBRs can be an advantage, since it provides alternatives, but also a challenge, since it implies a high degree of complexity in understanding, controlling, or simulating the process.

The most common production methods for microalgae are photobioreactors (PBRs) and ponds. A PBR allows for precise control of the environmental conditions required for optimum microalgae production [21]. The main differences between the two are the investments required and operational costs. PBRs require considerable investments and have high operational costs, the resulting microalgae being used for products with high value, such as pharmaceuticals, nutraceuticals, and cosmetics. In ponds, a higher quantity of biomass is produced for low-value products, with lower investments and operational costs.

The disadvantage of PBRs production method is a low scalability for production output, with high investment and operational costs [22]. Because of these barriers, it is difficult for companies to achieve profitability, technological innovations being essential for increased productivity [23]. The treated effluent from PBRs should meet the wastewater discharge and reuse standards [24]. The most significant limitation of cultivating microalgae in PBRs, with municipal wastewater as the growing medium, is the low settleability of microalgae, resulting in turbid effluents, a limitation that has been addressed in scientific papers from the past years. To improve the decantation of biomass, there are several PBR configurations, such as bioflocculation, biofilm PBR, aerobic granular sludge, and membrane PBR.

The number of scientific papers that tackle the use of PBRs to treat different types of wastewaters keeps on growing steadily. Thus, a comprehensive analysis of the existing literature regarding the use of microalgae to treat MWW in PBRs is necessary, in order to understand the current state of this research field, its advances, and opportunities.

The conventional nutrient removal, in a wastewater treatment plant, is represented by the biological process of nitrification and denitrification, where nitrification, in particular, consumes more than 50% of the total energy [25–27]. Thus, the integration of PBRs in the wastewater treatment process is of great scientific interest, representing a solution for a low-energy and environmentally friendly alternative for nitrogen and phosphorous removal, considering, at the same time, the legal requirements for effluents.

In PBRs, phosphorus is removed through precipitation and assimilation by microalgal biomass. Phosphorus precipitation represents an indirect removal mechanism at high pH values (pH > 8). Hu Y., 2017, highlighted the importance of Ca++ availability in phosphorus precipitation [28].

The nitrogen/phosphorus (N/P) ratio in wastewaters could influence the phosphorus removal by microalgae. An optimal N/P ratio has been observed between 5 and 12 for municipal wastewater [29]. This ratio can vary depending both on the species of microalgae and their phosphorus uptake capacity [30].

Another method of microalgae production is represented by pond culture. Upscaling outdoor microalgae production is limited by climate conditions, as cultures are exposed to external weather changes [31].

The current review article used data from scientific experiments that involved pilot scale studies or small laboratory set-ups for cultivating microalgae in MWW, with limited data on energy consumption or installation footprint.

2.2. Cultivation Types

Different microalgae strains are capable of performing differently, based on the growing medium and the desired outcome; thus, the proper strain must be selected in order to achieve the highest efficiency. Each species can have different metabolites, including hydrocarbons, fatty acids, vitamins, lipids, pigments, and antioxidants.

Mehariya et al., 2021, summarized the various wastewater treatment systems that used microalgae for biomass production and lipid synthesis. A microalgae culture with multiple applications (biomass and biofuel for example) can reduce the energy cost of production; reduce the greenhouse gases, since microalgae can use atmospheric CO_2 as a carbon source; and reduce the required fertilizer and water source [32].

Microalgae are very adaptable to various environmental conditions, with the capacity to adjust their metabolic activities. This adaptability can be used to classify microalgae as follows:

- autotrophs—light is used with inorganic carbon for photosynthesis (CO);
- heterotrophs—organic carbon is used as the carbon source, regardless of light supply;
- mixotrophs—both types of carbon source (photosynthesis and organic carbon source) are used [33].

2.2.1. Photoautotrophic Cultivation

The vast majority of articles selected for this review, that studied the efficiency of microalgae in treating municipal wastewater in photobioreactors, used phototrophic cultivation. This type of cultivation technique is also the most commonly used for producing large amounts of biomass, with considerable advantages, such as carbon neutrality and cost efficiency, making it currently the only economically feasible approach for large-scale production of microalgae biomass [32].

2.2.2. Heterotrophic Cultivation

Heterotrophic species of microalgae are much rarer than photoautotrophic species. Considering that organic compounds have an inhibitory effect on the growth of microalgae, even at low concentrations, the industrial applications are reduced, but with considerable potential [34]. One significant advantage this type of culture system has over photoautotrophic cultivation is the extremely high cell densities that can be achieved, since the microalgae development is independent of the light supply [34].

Heterotrophic culture could be performed in industrial-scale fermenters, with greater control over culture factors like pH, temperature, oxygen levels, and carbon supply, and with significant biomass increase and high lipid content in microalgal cells [34].

2.2.3. Mixotrophic Cultivation

This type of cultivation presents several advantages. Organic compounds as a source of nutrition plus a light source have a synergistic effect in order to maximize the microalgae growth rates. One limiting aspect of photoautotrophic culture is the high microalgal cell density achieved during growth, which reduces the amount of light that penetrates through the growing medium. Mixotrophic microalgae are capable of using the organic substrate as nutrition as well as performing photosynthesis, light energy no longer being a limiting factor for growth. This combination of photosynthesis and heterotrophy also happens in a diurnal cycle which reduces biomass loss.

Spirulina platensis cultivated in a mixotrophic system results in a microalgal biomass richer in carbohydrates, which can further be processed in bio-compounds such as lipids, proteins, vitamins, antioxidants, and pigments [35].

2.3. Microalgae and Nutrient Reduction in Municipal Wastewater

In Table A1, the results obtained in scientific papers that approached the topic of municipal wastewater treatment using microalgae in the last 7 years are summarized with an emphasis on the species of microalgae used, the initial concentrations of nitrogen/phosphorous/carbon, the growing conditions (light intensity, temperature, duration of the experiments, air source, cellular density), growing system (if it was mentioned), and the nutrient removal rates, accumulated biomass, and lipids/proteins measured in microalgae.

2.3.1. Nitrogen Removal

The most used species of microalgae in MWW treatment experiments from the selected papers in this review were *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Chlorella pyrenoidosa*. The nitrogen removal rates varied between 89 and 100% for microalgae, with slightly lower values for blue–green algae. Figure 4 presents the total nitrogen removal rates collected from scientific articles that approached municipal wastewater treatment with the use of microalgae. Factors that influenced this range of results are the concentration of initial nitrogen in MWW and the length of time the experiments took to conduct.

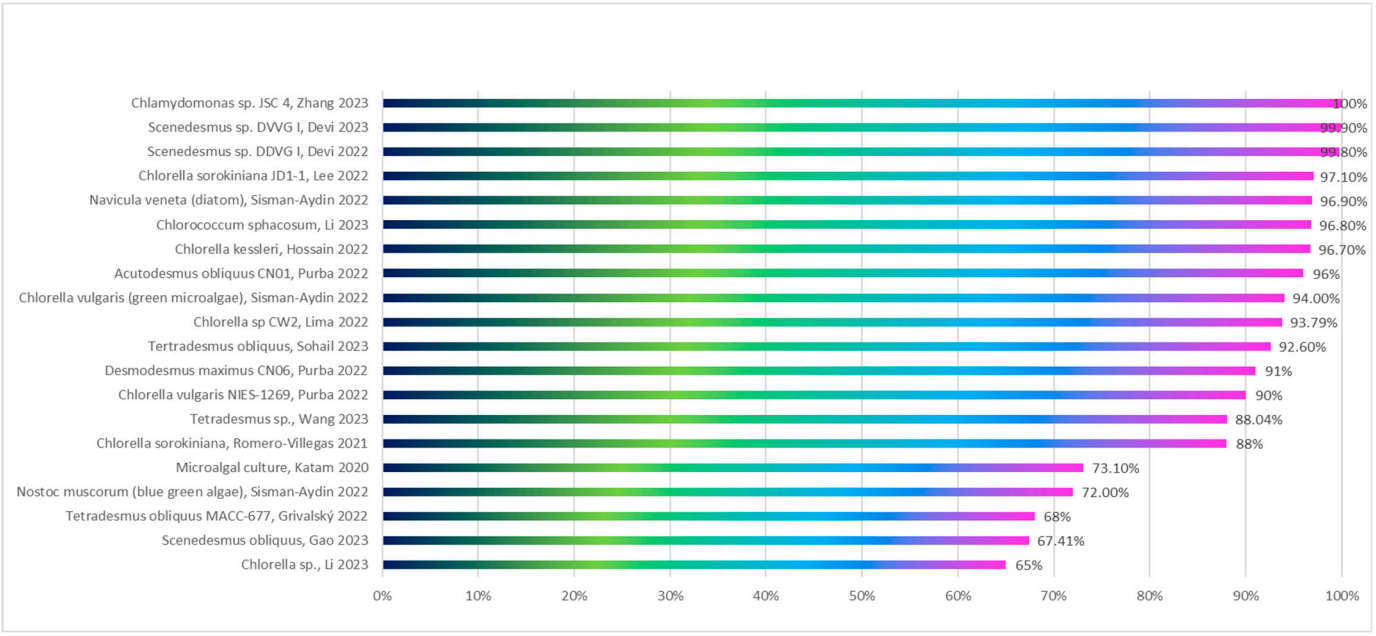

Figure 4. Total nitrogen removal rates obtained in scientific articles, for microalgae species cultivated in municipal wastewater [9,36–50]. Note: Most representative values were selected from the articles presented in Table A1.

Purba et al., 2022, obtained a 96% nitrogen removal efficiency, with complete NH_3-N removal for the species *Acutodesmus obliquus*, cultivated in MWW from a sewage treatment plant, in Kuala Lumpur, Malaysia [36]. In the same experiment, *Desmodesmus maximus* achieved a 91% nitrogen removal with 78% NH_3-N removal rate after 12 days. *Chlorella vulgaris* had the lowest nitrogen removal rate at 90% with 78% NH_3-N removal efficiency, cultivated in the same conditions. The initial total nitrogen was 29.2 ± 5 mg/L, and the initial NH_3–N was 14.9 ± 1.5 mg/L.

Sisman-Aydin et al., 2022, cultivated three types of organisms that are considered microalgae under mixotrophic conditions in wastewater from İzmir, Turkey [37]. Nostoc muscorum (a blue–green algae) had the lowest nitrogen (72.0%) and phosphorous (88.2%) removal rates, while Navicula veneta (a diatom) had the highest nitrogen (96.9%) and

phosphorous (99.8%) removal rates, with *Chlorella vulgaris* (a green microalgae) being close in efficiency (94.0% N removal and 98.6% P removal rates).

Sepehri et al., 2020, concluded that photooxygenation could replace aeration used in the conventional nitrification process, with a positive outcome, 100% of NH_4^+ being removed by *Chlorella vulgaris* at a low C/N ratio [51].

Chieti et al., 2024, used a microalgal consortium formed by *Auxenochlorella protothecoides*, *Tetradesmus obliquus*, and *Chlamydomonas reinhardtii* in different concentrations of wastewater media. Their results suggest that the consortium acclimated to the different growing media, by modulating the proportion of the species and their metabolism, with proficient nutrient removal efficiencies (close to 100%) [52].

Li et al., 2024, evaluated a microalgae consortium in a pilot-scale tubular PBR for municipal wastewater treatment, compared with an aeration column PBR. The highest removal rates for NH_4^+-N and TN were 99.86% and 92.75%, respectively, and the average TP removal rate was only approximately 25.69% [53].

Nitrogen cycles in microalgae are fundamental. Microalgae are capable of utilizing nitrogen from different inorganic (e.g., NH_4^+, NO_3^-, and NO_2^-) and organic sources (purines, urea, and amino acids). Many species prefer NH_4^+ because it is already a reduced form [54].

NH_4^+ is assimilated by membrane transporters known as the ammonium transporter. Once it has crossed the membrane, NH_4^+ is directly used to synthetize the amino acids needed for growth and other metabolic functions.

Ardo et al., 2024, cultivated microalgae in synergistic light intensity and photoperiod conditions (200 µmol photons/m^2s and 12 h light: 12 h dark, respectively), with removal rates for chemical oxygen demand (COD) and ammoniacal nitrogen both achieved at about 80% [55].

Figure 5 presents the removal rates for NH_4^+-N, collected from scientific articles that approached municipal wastewater treatment with the use of microalgae.

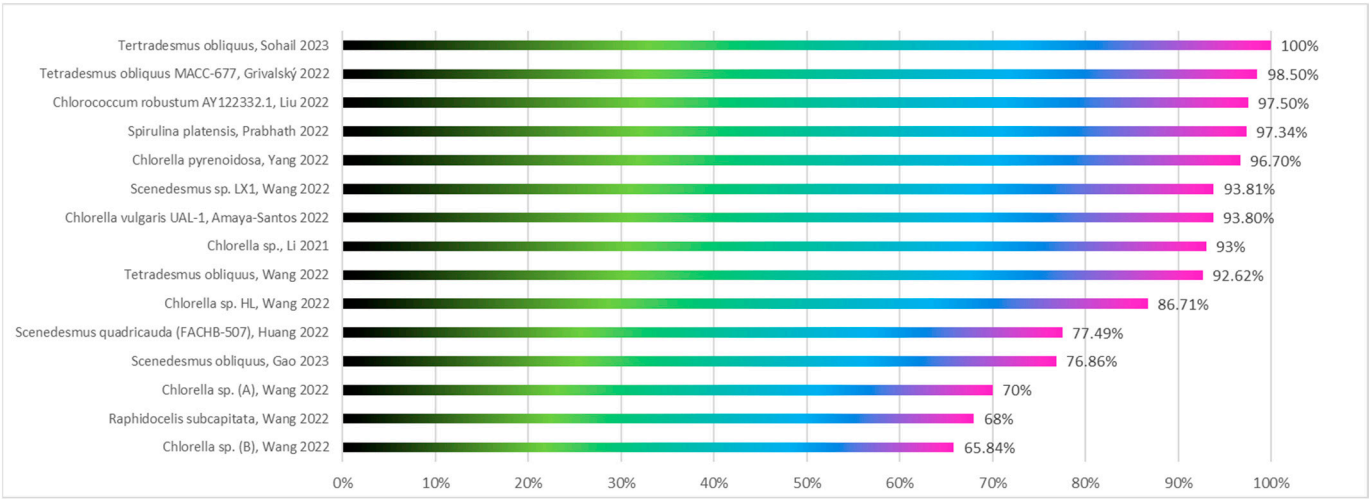

Figure 5. Total NH_4^+-N removal rates obtained in scientific articles, for microalgae species cultivated in municipal wastewater [39,43,49,50,56–61]. Note: Most representative values were selected from the articles presented in Table A1.

NO_2^- and NO_3^- can also be utilized, but they need to be reduced first [48]. Furthermore, NO_3^- transport into the cell is an energy-intensive mechanism that consumes ATP directly. The major drawback of NH_4^+ is the conversion into toxic NH_3 at higher pH, which often causes the autointoxication of cultures during photosynthesis. The conversion of NO_3^- and NO_2^- to NH_4^+ via microbial dissimilatory nitrate reduction is beneficial [62].

Consequently, in a microalgae culture that requires nitrification, either a denitrification stage in the treatment chain or a sufficiently long retention time is needed for the microalgae to decrease NH_4^+ significantly and for the nitrogen compounds to reach the required total nitrogen discharge limits [62]. However, both methods have the drawback of increasing operating costs and uncertainty. Various authors have recorded that in a steady-state algae–bacteria phase, approximately 60–85% of NO_3^- in the media is oxidized to NH_4^+, but only 13–40% is assimilated by microalgae [63]. Figure 6 presents the removal rates for NO_3^-, collected from scientific articles that approached municipal wastewater treatment with the use of microalgae.

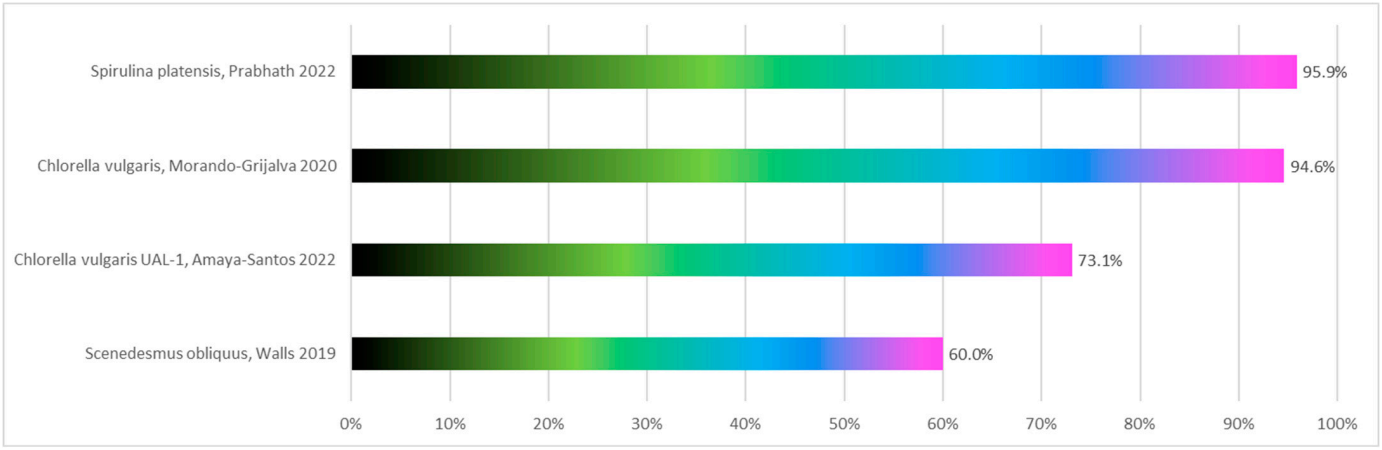

Figure 6. Total NO_3^- removal rates obtained in scientific articles for microalgae species cultivated in municipal wastewater [60,61,64,65]. Note: Most representative values were selected from the articles presented in Table A1.

2.3.2. Phosphorous Removal

Phosphorus is a key element involved in multiple biochemical pathways. It is a structural element of phospholipids, nucleotides, and an essential component of the biological energy currency ATP. Figure 7 presents the removal rates for total phosphorus, collected from scientific articles that approached municipal wastewater treatment with the use of microalgae.

Inorganic P is commonly considered the most bioavailable form, and microalgae preferentially assimilate $H_4PO_2^-$ via symporter channels with Na^+ or HPO_2^+ ions serving as the driving force [66]. Phosphorous is integrated into organic substances via ATP, which allows for the transition of the PO_4^{-3} group to organic compounds [62]. During P-rich conditions, microalgae store P as acid-insoluble polyphosphate granules for their metabolic needs for further use, which is called luxury uptake [67].

In the selected papers, phosphorous was removed in a wide range of rates. Morando-Grijalva et al., 2020, reported a removal rate for P of 97.9%, using *Chlorella vulgaris* [65]. Grivalský et al., 2022, reported that *Tetradesmus obliquus* MACC-677 had a removal rate for P of 51% [50]. The lowest removal rate for phosphorous (48.1%) was obtained by Díaz et al., 2024, by cultivating microalgae for wastewater treatment in a membrane PBR; however, the nitrogen removal rate reached 96.99% [68].

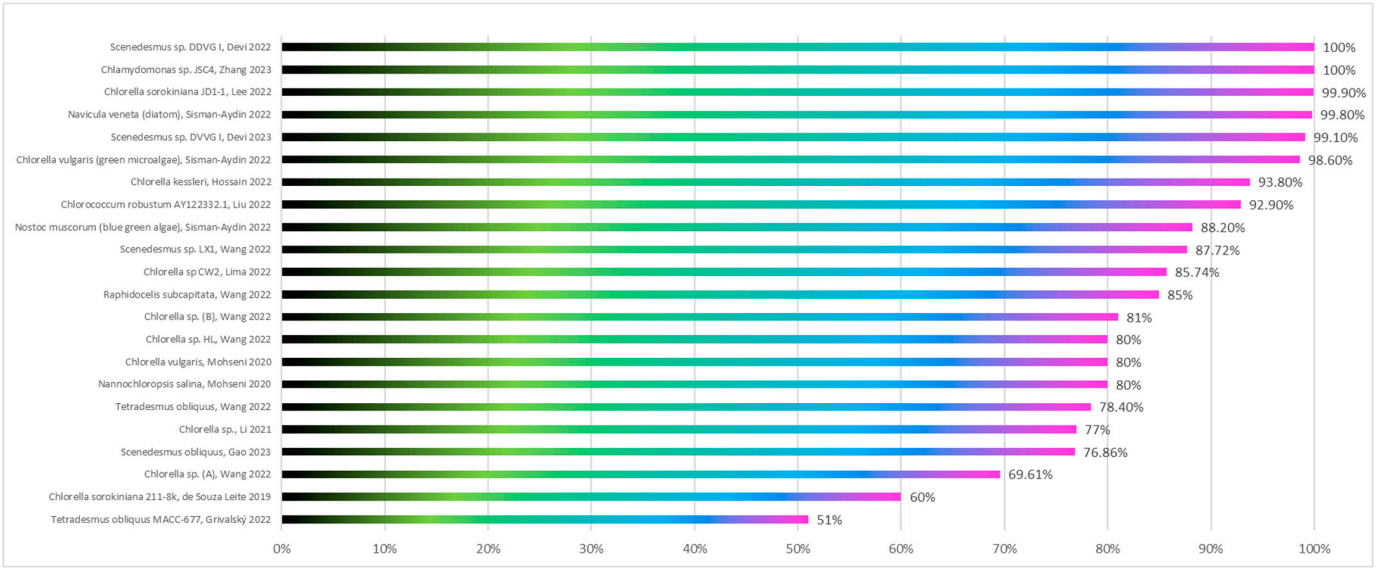

Figure 7. Total phosphorus removal rates obtained in scientific articles, for microalgae species cultivated in municipal wastewater [9,37,39–42,44,45,49,50,58,59,69,70]. Note: Most representative values were selected from the articles presented in Table A1.

2.3.3. Carbon Removal

During photoautotrophy, microalgae use CO and HCO_3^- for carbon fixation. In microalgae, the Calvin cycle provides carbon skeletons for multiple biochemical processes involving lipids and amino acids [71].

Microalgae can be grown as well in heterotrophic or mixotrophic systems, depending on the species. If the carbon sources in MWW are too complex, the growing medium could prove to be inhibiting to the proper development of microalgal biomass.

There is a lack of knowledge of the exact mechanism with which microalgae digest and assimilate more complex carbon compounds. While supplementing organic compounds with easily available carbon is a technique for improving the treatment efficiency of a microalgal WWT facility, it raises production costs.

Oss et al., 2022, used microalgae biomass as a precursor for activated carbon production, with urban wastewater as a growing medium. This feat goes to show the vast application microalgae biomass could be useful for a more sustainable future [72].

2.3.4. Lipid Production

During MWW treatment, microalgae can use the nutrients present in water in order to produce lipids. Increased lipid content and high biomass are normally difficult to achieve simultaneously. The usual approach is to focus on high biomass yield in the first stage of cultivation, after which microalgae are introduced in a stressful environment after harvesting. The stressors could be nutrient starvation, salinity, light reduction, or a combination of them [73].

Aketo et al., 2020, evaluated the efficacy of three microalgae species grown in wastewater for lipid production. *Parachlorella kessleri* NKG021201 showed high lipid productivity of 56 ± 1 mg/L/day and 35 ± 10 mg/L/day for *Chloroidium saccharophilum* NKH13, with 99% of N and 82% of P compounds removed from the wastewater by the strain NKG021201 [74].

Wang et al., 2021, obtained a lipid concentration of 51% in *Chlorella pyrenoidosa* cultivated in municipal wastewater using plant hormones [75].

He et al., 2024, applied an electric field on the growth and lipid production of microalgae in order to treat wastewater. In their study, the dry weight and lipid content of

microalgae increased by 47.45% and 28.28%, respectively, when optimized for light, growth phase, and energization time [76].

2.3.5. Protein Production

Microalgae biomass could represent a valuable alternative to conventional protein sources, especially for animal feed. Zhao et al., 2018, investigated different methods for separating polysaccharide, lipid, chlorophyll, and protein from *Chlorella* spp. The most efficient method removed 78.87% protein from the wet biomass while preserving the integrity of polysaccharides [77].

The highest protein content in microalgae biomass was measured by Wang et al., 2022, while growing *T. obliquus* in municipal wastewater, with protein representing 54.64% of the biomass [69].

3. Future Perspectives

The knowledge we have on PBRs spans more than 70 years of research and studies. Future research is needed to study not only the optimum conditions that can achieve the most efficient removal of nutrients and organic matter but also the employment of microalgae bioproducts in our daily life.

The most used solid–liquid separation operation in wastewater treatment is biomass separation by settling. Considering the reduced capacity of microalgae to settle, this is a considerable topic of research. New methods for improving this hinderance are studied, with combinations of membranes and PBRs expected to bring new results for biomass retention and effluent treatment.

Photosynthetic biofilms could be an alternative to facilitate biomass harvesting and processing. The application of biofilm-based reactors has shown potential for municipal wastewater treatment and the enhancement of microalgal biomass productivity.

On the other hand, Josa and Garfí, 2023, studied the social impacts of microalgae-based systems for wastewater treatment and bioproducts recovery, by comparing a system treating urban wastewater and another system treating wastewater from the food industry with a system for bioproducts production from microalgae grown in a standard growth medium. The recovered bioproducts considered in their study were as follows: natural pigments, biogas, and digestate (which can be reused as biofertilizer). Their results highlighted that microalgae grown in a standard medium had the lowest negative impact due to the simplicity of the system and the absence of contaminants, which consequently improves health and safety for workers; acceptability and olfactory impact for both consumers and the local community; and the presence of well-established legislation, regulatory frameworks, and full-scale deployment, which benefit value chain actors and society. This study plays an important role in identifying several social factors (health risks, societal acceptance, lack of social impact studies, awareness and education, cultural and psychological barriers), hindering a transition towards a circular bioeconomy in the microalgae-based systems for the wastewater treatment and resource recovery sector [78].

More studies are necessary for optimizing the design and operating technology, in order to implement these technologies at pilot or full scale.

4. Conclusions

The removal of nutrients and COD is mainly influenced by the interactions of microalgae and the growing-medium parameters, such as pH, light, temperature, dissolved oxygen, C/N/P ratio. More research is needed to understand the interactions between these factors and the removal mechanisms. The use of mathematical modelling in PBR systems has a high potential for their optimization, when correctly calibrated and validated.

The performance of PBRs for wastewater treatment at a large scale must be better studied, including the revalorization of biomass, the reuse of treated water, and the socio-environmental and economic implications.

The challenges of PBR use for municipal wastewater treatment (a high footprint and harvesting and biomass processing costs to produce high-value products), if solved, could transform PBRs into a prominent wastewater treatment technology that could compete with conventional systems.

This illustrates the promise of microalgae for low-cost WWT applications. Biomass derived from microalgae can also be utilized for uses ranging from biofuel to cosmetics. Although microalgae treatment is quite useful, improper handling can be hazardous. Proper operating protocols must be followed when employing microalgae for wastewater treatment.

As a future research direction, to promote the European Green Deal values, it is necessary to evaluate the environmental impact of microalgae production by considering the volume of water treated per day and the energy consumption of the production system.

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Appendix A

Table A1. Results obtained in scientific papers that approached the topic of municipal wastewater treatment using microalgae.

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Consortium of Oedogonium calcareum, Oedogonium pringsheimii, Klebsormidium	-Te Puke municipal WWTP -Rotorua municipal WWTP, both located in the Bay of Plenty Region on the North Island of New Zealand	16.9 mol photons/m^2day	18.8 °C	-TN 19.5 mg/L -DRP 4.3 mg/L	12 days	-	-	5 L plastic buckets filled to 4 L, with a surface area of 0.035 m^2	-Biomass 9.7 g DW/m^2day -Effluent TN 2.5 mg/L -Effluent DRP 1.8 mg/L -Protein 32.0%	[8]
Scenedesmus sp. *DVVG I*	Domestic sewage treatment plant (DSTP), located in Guwahati, India, with added urea to achieve the N/P ratio of N_{250}:P_5	Llight intensity of 81 µmol/m^2/s in a 12:12 light:dark regime	27 °C	-COD (mg/L) 1700 ± 1.9 -TN (mg/L) 576 ± 1.9 -TP (mg/L) 12.5 ± 1.9	12 days	Initial optical density (OD 680 nm) of 0.4	-	500 mL Flask with 250 mL of culture medium	-Cell harvesting efficiency (%) 0.02 -BC (g/L) 5.17 -BP (mg/L*d) 52.5 ± 1.1 -Lipid (%) 34 -Removal efficiency COD (%) 89.5 -Removal efficiency TN (%) 99.9 -Removal efficiency TP (%) 99.1	[9]
Chlorella pyrenoidosa	Wastewater network in Xili University Town, China	4000 lux Photoperiod of 24 h:0 h L/D	23.2 °C	-COD 104 mg/L -NH_4^+-N 26.3 mg/L -TN 33 mg/L -TP 0.6 mg/L -pH 7.8 -SS 105 mg/L	12 days	-	-	-	-Lipid yield was 0.18 g/L -dry weight 0.6 g/L -lipid content 25%	[10]
Scenedesmus obliquus (UTEX B2630) *Chlorella vulgaris (UTEX 259)* *Chlorella sorokiniana (UTEX 1230)*	Wastewater generated by a local beef packaging plant (Midwest, USA)	450 lux fluorescence light 16:8 light/dark	20 °C	-sCOD 798 mg/L -TN 132.5 mg/L -TP 116 mg/L	7 days	-	Ozone at flow rate of 3.3 L/min	-	-Biomass 2297.1 ± 393 mg/L -60.1% sCOD removed -63.4 ± 2.3% TN removed -77.6 ± 5.5% TP removed	[11]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Acutodesmus obliquus CN01	-AF6 medium initially -MWW from Sewage Treatment Plant, Kuala Lumpur, Malaysia	24 h white fluorescent lamp with light intensity of 80 µmol/m^2s	24 ± 2 °C	COD130 ± 44 mg/L TN 29.2 ± 5 mg/L TP 4.8 ± 0.3 mg/L NH_{22}–N 14.9 ± 1.5 mg/L NO_2–N 3 ± 1.7 mg/L NO_3^-–N 13.3 ± 5.5 mg/L pH 7.2 ± 0.5	12 days	50 mg/L in in 300 mL medium	Continuos aeration with 0.1 L/min flowrate	-aerated flasks	-Biomass accumulation at 617.5 mg/L in MWW -Biomass accumulation at 540 mg/L in AF6 medium -N removal efficiency of 96% -complete NH_{22}–N removal -Lipid content in AF6 medium 31.45%	[36]
Desmodesmus maximus CN06									-Growth rate at 0.23/day MWW -Growth rate of 0.28/day in in AF6 medium -Biomass accumulation at 785 mg/L in AF6 medium -Biomass accumulation at 705 mg/L in wastewater -N removal efficiency of 91% -NH_3–N removal efficiency at approximately 78% -Lipid content in wastewater 57.82%	
Chlorella vulgaris NIES-1269									-Biomass accumulation at less than 500 mg/L after 12 days of cultivation period in both media -N removal efficiency of 90% -NH_3–N removal efficiency at approximately 78% -Lipid content in wastewater 46.38%	
Nostoc muscorum (blue green algae)	-f/2 and BG11 medium enrichment -Wastewater from İzmir, Turkey	L:D = 16: 8 (mixotrophic condition) -Day-light fluorescent illumination, with 100 µmol m^{-2} s^{-1} light intensity	20 ± 0.5 °C	COD 400 ± 12.2 mg/L N 60.98 ± 0.2 mg/L P 6 ± 0.41 mg/L	7 days	0.86 mg/L Chl-a in each trial group	Airflow rate about 400 mL/min	1 L batch reactors	-85.7% COD removal -72.0% N removal -88.2% P removal -growth rates 0.842/day	[37]
Navicula veneta (diatom)									-95.7% COD removal -96.9% N removal -99.8% P removal -growth rates 0.805/day	
Chlorella vulgaris (green microalgae)									-91.8% COD removal -94.0% N removal -98.6% P removal -growth rates 0.833/day	

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Tetradesmus sp. 99.68%	Rare earth tailings wastewater—Ganzhou City, Jiangxi Province	White LED lamp, length 1 m, spectrum between 450 and 460 nm, light intensity 150 µmol/m^2s	Ambient temperature (20–35 °C)	C/N = 0.51–0.56 High NH_4^+-N (100 ± 30 mg/L) Weak alkalinity (pH < 9.5)	140 days	-	Mechanical stirring of 20–30 r/min	-Cylindrical container made of acrylic plate -250 mm in diameter, 1 m in height and covers an area of 0.4 × 0.4 m^2 -Working volume of 50 L	-88.04% TN removed -TOC increased with the reduction of NH_4^+-N -pH gradually decreased to 6 after 2–2.5 days	[38]
C. vulgaris (NIES-2170)	-Effluent of the final sedimentation tank of a domestic wastewater treatment plant in Kanazawa, Ishikawa, Japan, (RTW) -Synthetic treated wastewater (STW)	Light with fluorescent lamps 2500 lx.	25 °C	STW -$NaHCO_3$ 210 mg -$NaH_2PO_4 \cdot 2H_2O$ 1.51 mg -NH_4Cl 57.3 mg -$CaCl_2$ 70 mg -$MgSO_4 \cdot 7H_2O$ 40 mg -PIV metals 3 mL -Vitamin solution 0.1 mL -Distilled water 997 mL	40 days	-	Ambient air	2 L glass bottle (136 × 265 mm, mouth size 30 mm), capped with a silicone plug with three ports.	-Suspended solids concentration reflects the microalgal growth 708 mg/L -Carbohydrate concentration 62.6 ± 23.3 mg/L -Biomass productivity 0.03 g/Day	[79]
Nannochloropsis gaditana (CCAP 849/5) -*Chlorella sorokiniana* (CCAP 211/11k) -*Chlorella* sp. -consortium of *Chlorella* sp. and *Dunaliella tertiolecta*	Municipal treatment plant Palermo, Italy	-	-	-	15 days	-	-	500 mL cultivation flask	-TN removal rate measured: 36.4%, 77.3%, 76.4% and 88.2% for *N. gaditana*, *C. sorokiniana*, *Chlorella* sp. and the consortium -TP was removed by 61.0%, 56.1%, 17.1% and 58.5% in cultures of *N. gaditana*, *C. sorokiniana*, *Chlorella* sp. and the consortium	[80]
Micractinium sp. with less *Scenedesmus* sp.	Wastewater treatment system at Kingston on Murray in the South Australian Riverland	-	-	-	-	-	-	Flocculation HRAP	-29.45% of ammonia removed -47.11% of total Kjeldahl nitrogen removed -97.03% of total phosphorus removed	[81]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Nephroselmis sp. *KGE2*	Livestock wastewater effluent from Jeongeup in the Republic of Korea	White fluorescent light with an illumination intensity of 100 µM photon/m^2s	25 °C	-COD (mg/L) 269.6 ± 4.0 -pH 8.5 ± 1.3 -TN (mg/L) 145.0 ± 2.0 -NO_2 (mg/L) 59.4 ± 1.2 -NO_3 (mg/L) 14.9 ± 1.1 -TP (mg/L) 4.5 ± 0.5 -SO_4^2 (mg/L) 17.4 ± 1.3 -Ca^{2+} (mg/L) 31.8 ± 1.9 -Cu^{2+} (mg/L) 4.6 ± 0.1 -Fe^{2+} (mg/L) 8.6 ± 0.1 -Mg^{2+} (mg/L) 1.4 ± 0.3 -Zn^{2+} (mg/L) 3.6 ± 0.1	20 days	Initial microalgae conc. of 0.05 g/L	-	Closed 500 mL serum bottles with a rubber cap	-Biomass content (g/L) 0.45 ± 0.06 -Specific growth rate (/day) 0.09 ± 0.01 -Cell doubling time (day) 7.33 ± 0.59 -TN uptake per biomass (mg/g) 75.37 -TP uptake per biomass (mg/g) 2.84 -Lipid productivity (g/L day) 0.13 ± 0.02	[82]
Autodesmus obliquus KGE17									-Biomass content(g/L) 0.82 ± 0.10 -Specific growth rate (/day) 0.11 ± 0.01 -Cell doubling time (day) 5.96 ± 0.74 -TN uptake per biomass (mg/g) 60.98 -TP uptake per biomass (mg/g) 5.67 -Lipid productivity (g/L day) 0.13 ± 0.02	
Chlorella sorokiniana UUIND6	Municipal wastewater from Prem Nagar sewer system, Dehradun, Uttarakhand, India	White-light LED	25 ± 2 °C	-Colour dark tan -pH—7.65 ± 0.1 -Odour—Yes -Alkalinity mg/L 938.35 ± 11.5 -Hardness mg/L 1523.66 ± 22.0 -Total Kjeldahl N mg/L 33.97 ± 1.5 -Inorganic P mg/L 18.03 ± 0.5 -TOC mg/L 11.03 ± 0.6 -Dissolved O mg/L 1.16 ± 0.1 -BOD mg/L 15.15 ± 0.8 -COD mg/L 45.22 ± 0.8	14 days	OD 730 nm	-	Erlenmeyer	-Colour—Clear -pH—8.43 ± 0.1 -Odour—No -Alkalinity mg/L 313.33 ± 10.4 -Hardness mg/L 323.66 ± 12.4 -Total Kjeldahl N mg/L 1.56 ± 0.1 -Inorganic P mg/L 0.52 ± 0.1 -Total organic C mg/L 3.28 ± 0.1 -Dissolved O mg/L 5.03 ± 0.2 -BOD mg/L 2.76 ± 0.2 -COD mg/L 7.38 ± 0.1 -Lipid content 19.7 ± 1.3%	[83]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorella pyrenoidosa	-BG11 medium -local domestic wastewater treatment plant	120 µmol/m^2/s light intensity with 16:8 h light and dark as photoperiod	25 ± 2 °C	-pH 6.5 ± 0.23 -Colour cloudy -Odour Unpleasant -Temperature (°C) 18.3 ± 1.34 -Total dissolved Solid (mg/L) 612.2 ± 12.34 -Electrical Cond. (µS/m) 512.31 ± 20.23 -Salinity (mg/L) 0.43 ± 0.012 -COD (mg/L) 335.43 ± 15.5 -Dissolved oxygen (mg/L) 2.31 ± 0.32 -BOD (mg/L) 111.34 ± 13.2 -Alkalinity (mg/L) 312.44 ± 4.65 -Nitrate (mg/L) 25.653 ± 0.54 -Nitrite (mg/L) 1.23 ± 0.09 -Phosphate (mg/L) 7.81 ± 0.73 -Ammonia (mg/L) 36.4 ± 3.33	15 days	-	-	1 L Erlenmeyer flask with a working vol. of 500 mL	-Biomass BG11 1.54 g/L -Biomass WW 0.71 g/L -Protein content BG11 46.65% -Protein content WW 34.43% -Carbohydrate content WW 33.21% -Lipid content WW 25.34%	[84]
Scenedesmus obliquus									-Biomass BG11 1.25 g/L -Biomass WW 0.90 g/L -Protein content BG11 50.34% -Protein content WW 35.76% -Carbohydrate content WW 30.32% -Lipid content WW 25.67%	
Chlorella sorokiniana									-Biomass BG11 1.45 g/L -Biomass WW 0.76 g/L -Protein content BG11 44.12% -Protein content WW 33.35% -Carbohydrate content WW 32.32% -Lipid content WW 26.23%	
Chlorella vulgaris	WWTP in Ziyang city	Light intensity was ranged from 1760 lx to over 20,000 lx	-	-NH_4-N (mg/L) 16.60 ± 4.53 -TN (mg/L) 21.87 ± 5.18 -TP (mg/L) 1.65 ± 0.46 -COD (mg/L) 141.41 ± 32.93 -DO (mg/L) 6.83 ±1.51 -pH 8.21 ± 0.50	-	-	Aeration rate was 200 mL/min in 12 h	Aeration columnar PBRs with working volume of 1.2 L	-NH_4-N (mg/L) 5.56 ± 5.84 -TN (mg/L) 8.19 ± 5.58 -TP (mg/L) 0.27 ± 0.37 -COD (mg/L) 44.11 ± 30.55 -DO (mg/L) 8.03 ± 1.91 -pH 8.35 ± 0.52	[85]
Chlorella vulgaris	Municipal sewage generated from a college campus	Up 100,000 Lux	22 °C to 27 °C	-pH (µS/cm) 8.41 -Total dissolved solids 485 (mg/L) -COD 920 (mg/L) -BOD 198 (mg/L) -Alkalinity as $CaCO_3$ 200 (mg/L) -Chlorides 134.7 (mg/L) -Nitrates 52 (mg/L) -Sulphates 75 (mg/L) -Phosphates 18 (mg/L) -Potassium 28 (mg/L)	4 months	-	-	50 L capacity outdoor open syntax tank	-0.67 g/L biomass -0.26 g/L lipid -Nitrates reduction 93% -COD reduction 95% -BOD reduction 92% -pH (µS/cm) 7.5 -Chlorides 21.3 (mg/L) -Sulphates 10 (mg/L) -Phosphates 4 (mg/L)	[86]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Amphora sp. *RRSE1*	MWW from the Sewage Treatment Plant in Coimbatore, Tamil Nadu, India	-	30 °C	-pH 7.21 -Conductivity (mS/cm) 1864 -TDS (ppm) 931 -Chloride (mg/L) 420.46 -Nitrate (ppm) 59 -Phosphate (ppm) 4 -Na (ppm) 233 -K (ppm) 32.6 -Ca (ppm) 34.1	14 days	10% *v*/*v* cells	-	500 mL Erlenmeyer flasks containing 200 mL media	-Specific growth rate 0.025 -Doubling time 0.096 -Generation time 10.41 -Biomass productivity (mg/L/day) 0.23 ± 0.04 -Lipid content (%) 49.28 ± 1.53 -Lipid productivity (mg/L/day) 0.114 ± 0.07	[87]
Scenedesmus obliquus	Domestic wastewater from the University Town of Shenzhen	2500–3000 lx	23.2 °C	CODcr (mg/L) 230–250 TN (mg/L) 40–65 NH_4-N (mg/L) 24–63 NO_3-N (mg/L) 1–4 TP (mg/L) 4.3–5.5 pH 6.8–7.0	18 days	1×10^5 Cells/mL	-	1 L conical flask with 550 mL of WW	-Lipid yield 0.36 g/L -Lipid content 23.8%	[88]
Chlorella vulgaris	Sewage treatment plant treating low-strength MWW from Okhla, India	11,000 Lux With 12 h light: dark cycle	25–28 °C	-COD (mg/L) 107 ± 4 -NO_3^--N (mg/L) 14 ± 1.55 -NH_4^+-N (mg/L) 46.3 ± 0.02 -PO_4^{3-}-P (mg/L) 15.2 ± 1.22 -pH 7.55 ± 0.01	3 days	OD 0.2 at 680 nm	1.5 L/min air	1 L cylindrical vessel	-COD (mg/L) 60.1 ± 1 -NO_3^--N (mg/L) 1.2 ± 0.2 -NH_4^+-N (mg/L) 0.6 ± 0.1 -PO_4^{3-}-P (mg/L) 5.5 ± 0.5 -Biomass 1 g/L	[89]
C. pyrenoidosa									-COD (mg/L) 54 ± 3.2 -NO_3^--N (mg/L) 1.5 ±0.1 -NH_4^+-N (mg/L) 5.4 ± 0.1 -PO_4^{3-}-P (mg/L) 1 ± 0.1 -Biomass 1.4 g/L	
C. minutissima									-COD (mg/L) 51 ± 2.7 -NO_3^--N (mg/L) 2.4 ± 0.3 -NH_4^+-N (mg/L) 7.3 ± 0.3 -PO_4^{3-}-P (mg/L) 4.8 ± 0.6 -Biomass 1.4 g/L	
Spirulina sp.									-COD (mg/L) 47 ± 2.4 -NO_3^--N (mg/L) 3.8 ± 0.6 -NH_4^+-N (mg/L) 6.7 ± 0.3 -PO_4^{3-}-P (mg/L) 3.6 ± 0.1 -Biomass 1.4 g/L	
Chroococcus sp.									-COD (mg/L) 56 ± 0.2 -NO_3^--N (mg/L) 1.6 ± 0.1 -NH_4^+-N (mg/L) 7.6 ± 0.1 -PO_4^{3-}-P (mg/L) 4.6 ± 0.1 -Biomass 1.1 g/L	

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Scenedesmus obliquus	Wastewater Treatment Plant Shenzhen, China	7000 lux; With 12 h light: dark cycle	-	-COD 240 mg/L -TP 50.02 mg/L -TN 141.26 mg/L -NH_4^+-N 130.96 mg/L -PO_4^{3-}-P 44.96 mg/L -pH 8.0	10 days	OD_{680} =0.10	Aeration rate 50 mL/min	Cylindrical PBRs (0.8 L)	-Utilization rate TN (%) 67.41 ± 0.66 -Utilization rate TP (%) 76.86 ± 1.50 -Utilization rate NH_4^+-N (%) 76.86 ± 0.70 -Utilization rate PO_4^{3-}-P (%) 81.70 ± 1.61 -OD680 = 0.6	[39]
Chlorella pyrenoidosa	WW Harbin Institute of Technology, Shenzhen, China	4000 lx 12 h dark/light photoperiod	25 ± 1 °C	-COD 240 ± 10 mg/L -TN 50 ± 10 mg/L -NH_4^+-N 40 ± 15 mg/L -TP 5.0 ± 0.5 mg/L -pH 7.0 ± 0.2	6 days	-	-	microalgae-wastewater system was 3 L	-Biomass (g/L) 0.6167 -Lipid production (g/L) 0.1083	[90]
Chlorella vulgaris	Simulated MWW: Sucrose (1500 mg/L), $FeCl_3 \cdot 6H_2O$ (10 mg/L), $CaCl_2$ (42 mg/L), NH_4Cl (226 mg/L), KH_2PO_4 (35 mg/L), $NaHCO_3$ (354 mg/L), K_2HPO_4 (180 mg/L), $MgCl_2$ (49 mg/L)	60–70 µmol/m^2s	25–28 °C	-NH_4^+-N 48 mg/L -COD 1800 mg/L -pH 7.6	7 days	-	Air supplied at a rate of 0.4 L/min	500 mL Erlenmayer flasks	-COD removal 70–72% -Ammoniacal nitrogen removal 57%	[91]
Chlorella pyrenoidosa	MWW well in Xili University Shenzhen, Chin	8000–80,000 lx	22–28 °C	-COD_{CR} 190–230 mg/L -TP 4.5–5.6 mg/L -TN 40–60 mg/L -NH_3-N 20–35 mg/L -pH 6.6–7.6	7 days	OD_{680} = 0.1	-	PBR with a diameter of 5 cm, a length of 50 cm and a volume of 1 L with 600 mL settled MWW	-Biomass 0.5 g/L -Lipid yield 0.1 g/L -Lipid content 26%	[92]
Chlorella kessleri	Secondary effluent of MWW treatment plants	L:D of 24:0 Light intensity of 65 µmol/m^2s	29 °C	TN = 41.2 mg/L TP = 8.87 mg/L	-	2.2 × 10^7 cells m/L	4% CO_2 and 96% air	-PBR made of PYREX -1 L Erlenmeyer flasks with working volume of 500 mL	-Removal efficiency TN = 96.7% TP = 93.8% -Specific growth rate 0.43/day -Biomass productivity 56 mg/Ld	[40]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorella vulgaris	Synthetic wastewater	light intensity of Li-250 A photometer	30 °C	-	12 days	-	-	-	-Lipid content at 40% salinity 23.5% -Lipid productivity at 40% salinity 48.5 mg/Ld -Biomass 0.64 g/L	[93]
Wastewater-adapted microalgae/Chlorella vulgaris	Wastewater (non-aerated) from Parkandabad treatment facility, Mashhad, Iran	-3500 lux -40 µmol photon/m^2s 16:8 h light-dark	25 °C	-Phosphate content 8.5 mg/L -TN 133.2 mg/L -Kjeldal nitrogen 129 mg/L -nitrate 4.2 mg/L -COD 664 mg/L	-	-	-	PBR with 2.7 L working volume	Lipid contents -*Chlorella vulgaris* 27.5% DCW -Wastewater-adapted microalgae 26% DCW	[94]
Chlorella pyrenoidosa	Synthetic wastewater	102.6–110.5 µmol photon/m^2s	25 °C	-Dissolved inorganic N 40.0 mg/L -Dissolved inorganic P 5.0 mg/L -Added salinity of 0% (control group), 0.5%, 1.0%, 2.0%, and 3.0%	83 days in successive cycles of 24 h	180 mg/L	Gas flow 40 mL/min	Cylindrical PBR volumes of 1.5 L	-DCW (dry cell weight) 1.46 g/L -Biomass production 53.62 mg/Ld at salinity 0.5% -Removal rate of NH_4^+-N 96.7% -Removal rate of DIN 95.4% -Removal rate of DIP 97.7% -Lipid content 65.2% at salinity was 3% -Lipid productivity 22.50 mg/Ld at salinity 1%	[56]
Scenedesmus quadricauda (FACHB-507)	Simulated domestic wastewater	3200 lux of light intensity; 12/12 h light/dark	20 °C	-COD 100 mg/L (dextrose monohydrate) -NH_4^+-N 25 mg/L (NH_4Cl as N source) -PO_4^{3-}-P 3 mg/L (K_2HPO_4 as P source)	10 days	0.1 g/L	-	self-designed oscillating grid reactor	-Biomass 0.294 g/L -specific growth rate 0.47/d -Removal efficiency of COD 82.73% -Removal efficiency of NH_4 nitrogen 77.49% -Removal efficiency of phosphate 65.17% -Polysaccharide content 8% -Polysaccharide yield 1.75 mg/Ld -Protein content 26.04% -Protein yield 5.91 mg/Ld -Lipid content 17.91% -Lipid yield 5.27 mg/Ld	[57]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Consortium of indigenous microalgae (3 strains of Chlorella Sp., *2 strains of Desmodesmus* sp., *1 strain of Tribonema* sp.)	MWW	85 µmol photons/m^2s 12 h:12 h light:dark photoperiod	20 °C	-pH 7.09 -PO_4-P 7.55 mgP/L -NO_3 0.24 mgN/L -NH_4 45.3 mgN/L -TN 51.8 mgN/L -COD 255 mg/L	3 days	-	continuous supply of 4% CO_2	Cylindrical glass bioreactors (2 L, d = 130 mm, H = 200 mm)	-Ammonium removal 96 ± 2.1% -Total nitrogen removal 63.9 ± 3% -Phosphorus removal 99 ± 1%	[95]
Consortium of indigenous microalgae (3 strains of Chlorella Sp., *2 strains of Desmodesmus* sp., *1 strain of Tribonema* sp.)	Real MWW effluent of the primary clarifier at Seevetal WWTP	140 ± 3 µmol photons/m^2s 12:12 h (light:dark)	20 °C	-pH 7.12 -PO_4-P 7.26 mgP/L -NO_3 0.31 mgN/L -NH_4 52.2 mgN/L -TN 58.1 mgN/L -COD 242 mg/L	3 days	500–600 mg DW/L	-	Cylindrical glass bioreactors 2L	-TN removal 88.3 ± 13.9% -TP removal 98.2 ± 1.6% -COD removal 85.4 ± 3.4%	[96]
Chlorella sp. *CW2*	Municipal sewage coming from AMAP plant of Balestrate, Italy	127 µE/m^2s photon flux with a photoperiod light/dark of 12 h	27 °C	-COD 360 mg/L -TN 42 mg/L -TP 3.4 mg/L	10 days	-	-	500 mL Erlenmeyer flasks placed in an oscillating incubator	-Removal of COD 71.48 ± 0.7% -Removal of TN 93.79 ± 0.08% -Removal of TP 85.74 ± 2.21% -Lipid content 31.77 ± 2.5% -Carbohydrate content 34.04 ± 8.46%	[41]
Chlorella sorokiniana JD1-1	-Public wastewater treatment plant (Mokpo, Korea) -Public wastewater treatment plant (Yeongam, Korea)	50–100 µmol/m^2s	-	-pH 9.10–9.41 -TOC 12.7–17.2 (mg/L) -TN 30.3–71.3 (mgN/L) -TP 1.5–12 (mgP/L)	-	-	Air supply at a rate of 1 L/min	Glass bubble column PBR 3L	-Specific growth rate/d, 0.128–0.154 -Biomass concentration 640–1050 mgDCW/L -TN removal 97.1% -TP removal 99.9% -Lipid content (% of D.W.) 8.3 ± 0.1 -Lipid productivity (mg/L/d) 4.7	[42]
Desmodesmus subspicatus BG3-2									-Specific growth rate /d, 0.109–0.170	
Chromochoris zofingiensis BG3-18									-Specific growth rate /d, 0.066–0.086	
Tetradesmus obliquus HNIBR1									-Specific growth rate /d, 0.093–0.155	
Chlorodium ellipsoideum DB1-2									-Specific growth rate /d, 0.099–0.128	

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorococcum robustum AY122332.1	Secondary sedimentation tank of the Nanchang Xiaolan WWTPs, Nanchang, China	3000 Lux	25 °C	-pH 6.7 -NH_4^+-N mg/L 11.5–12.3 -TP mg/L 1.87–2.07 -IC mg/L 61.79–65.19 -TOC mg/L 10.34–11.54	20 days/40 days	0.5 g dry weight/L	-	Continuous-flow microalgae-bacteria tandem-type reactor, with a working volume of 4.5 L	-97.5% removal rate of NH_4^+-N -92.9% removal rate of P -TOC in final effluent 9.69 mg/L -10 g DW/L after 20 days	[58]
Scenedesmus abundans	Wastewater treatment plant of SSN College of Engineering, Kalavakkam, Tamil Nadu, India	Continuous fluorescent illumination of 4000 lux	28 ± 2 °C	-782.42 mg/L COD -262.07 mg/L NH_4^+ -48.8 mg/L NO_3^- -310.87 mg/L DIN -42.19 mg/L DIP	16 days	0.2 g/L	Air mixed with 5% CO_2	Lab-scale hybrid loop airlift PBR, working volume of 5.5 L	-Biomass 3.55 g/L -Productivity 209 mg/Ld -COD removal 80.19% -90.73% DIN removal -86.31% DIP removal -Protein 43.6 wt.% -Lipid 33.8 wt.% -Carbohydrate 20.15 wt.%	[97]
Chlorella sorokiniana 211-8k	WWTP in São Carlos, São Paulo State, Brazil	196 µmol/m^2s photoperiod of 16:8 day:night	30 °C	-pH 7.5 -Alkalinity 1620 -BOD5 207 mg/L -COD 547 mg/L -sCOD 262 mg/L -TS 1286 mg/L -TVS 414 mg/L -TN 300 mg N/L -TP 20 mg P/L	7 days	-	Aeration rate of 0.6 vvm	Flat panel PBRs	-Alkalinity removal 1.3% -BOD5 removal 94.3% -COD removal 92.2% -sCOD removal 81.9% -TS removal 65.6% -TVS removal 88.2% -DW 1 g/L -Biomass production 130 mg/Ld -Average specific growth rate 0.29/d -TN removal 100% -TP removal 60%	[69]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Scenedesmus sp. *LX1*	Domestic wastewater treatment plant in Beijing, China	28 μmol/m^2/s Light/dark cycle of 12/12	25 °C	-COD (mg/L) 548.31 ± 130.99 -TN (mg/L) 54.24 ± 5.75 -NH_4^+-N (mg/L) 50.97 ± 8.03 -TP (mg/L) 0.85 ± 0.10 -pH 8.16 ± 0.05	10 days	5×10^5 cells/mL	-	250 mL glass Erlenmeyer flasks	-Cell density $(5.26 \pm 0.61) \times 10^6$ cells/mL -COD removal 62% -NH_4^+-N removal 93.81% -TP removal 87.72% -Lipid 16.25%	[59]
Chlorella sp. *HL*									-Cell density $(2.44 \pm 0.12) \times 10^7$ cells/mL -COD removal 21% -NH_4^+-N removal 86.71% -TP removal 80% -Polysaccharides 4.25%	
R. subcapitata									-Cell density $(3.83 \pm 0.63) \times 10^6$ cells/mL -COD removal 79.01% -NH_4^+-N removal 68% -TP removal 85% -Polysaccharides 1.26%	
T. obliquus									-Cell density $(7.96 \pm 0.89) \times 10^6$ cells/mL -COD removal 65% -NH_4^+-N removal 92.62% -TP removal 78.40% -Protein 54.64% -Lipid 25.40%	
Chlorella sp. *(A)*									-Cell density $(1.08 \pm 0.15) \times 10^7$ cells/mL -COD removal 18.93% -NH_4^+-N removal 70% -TP removal 69.61%	
Chlorella sp. *(B)*									-Cell density $(1.34 \pm 0.02) \times 10^7$ cells/mL -COD removal 35.52% -NH_4^+-N removal 65.84% -TP removal 81% -Protein 35.30%	

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Desmodesmus communis (CCAP 276/4B)	-Wastewater from a treatment plant (WWTP) in Roja (Latvia) -Both primary (after primary settling) and secondary (after biochemical oxidation and secondary settling) wastewater used	100 µmol/m^2s and 16:8 h lighting regime	24–27 °C	Primary WW -TN 110 mgN/L -NH_4 68 mgN/L -NO_2^{+3} 21 mgN/L -TP 36 mgP/L -PO_4 30 mgP/L -pH 8.3 -EC 1700 µS/cm -BOD 530 mgO_2/L -COD 970 mg/L -N/P ratio 3:1	10 days	0.05 g DW/L	No additional aeration and CO_2 supply	1000 mL Pyrex® bottles	-Biomass productivity 0.047 gDW/Ld -DIP removal 99% -DIN removal 20.5%	[98]
Tetradesmus obliquus (CCAP 276/10)				Secondary WW -TN 40 mgN/L -NH_4 0.5 mgN/L -NO_2^{+3} 32 mgN/L -TP 36 mgP/L -PO_4 30 mgP/L -pH 8.2 -EC 1600 µS/cm -BOD 5.3 mgO2/L -COD 42 mg/L -N/P ratio 1:1					-Biomass productivity 0.038 gDW/Ld -DIP removal 99% -DIN removal 25.5%	
Chlorella protothecoides (CCAP 211/ 10C)									-Biomass productivity 0.049 gDW/Ld -DIP removal 93.9% -DIN removal 14.5%	
Tetradesmus obliquus	MWW collected from NUST, Islamabad	80 ± 5 µmol/m^2s light/dark cycle of 14:10 h	-	-pH 8.1 -Turbidity 16 NTU -EC 1.5 mS/cm -NH_4^+-N 47 ± 0.57 mgN/L -TKN 99 ± 0.9 mgN/L -PO_4^{3-}-P 9.1 ± 0.03 mgP/L -Ca 72 mg/L -Mg 45 mg/L -Pb N/D -Zn 0.30 mg/L -Mn 0.01 mg/L -Fe 0.11 mg/L -Cu 0.05 mg/L	14 days	Initial DW biomass density of 3 g/m^2	-	Lab-scale twin layer cultivation system	-Specific growth rate 0.17/d -Doubling time of 4.05 days -C capture rate 1.7 g/m^2d -NH_4^+-N removal 100% -TKN removal 92.6% -PO_4^{3-}-P removal 100% -Lipid content 30.3% -Lipid productivity 1.12 g/m^2d	[43]
Chlorella pyrenoidosa FACHB-5	MWW treatment plant located in Zhoushan, China	82.4–90.6 µmol photon/m^2s	30 °C	-NH_4^+-N 30.51 ± 2.81 mg/L -NO_2^--N 0.15 ± 0.03 mg/L -NO_3^--N 1.36 ± 0.22 mg/L -TN 53.92 ± 2.57 mg/L -BOD_5 152.35 ± 18.24 mg/L -COD 317.26 ± 15.39 mg/L -TOC 136.80 ± 5.33 mg/L -TC 187.68 ± 13.01 mg/L -PO_4^{3-}-P 3.81 ± 0.62 mg/L -TP 4.90 ± 0.38 mg/L -pH 7.26 ± 0.07	45 days	-	40 mL/min	AnMBR-MPBR hybrid system	-Biomass production rate 91.10 mg/Ld -Carbon content 0.47 g/g dry biomass -Nitrogen content 0.140 g/g dry biomass -Phosphorus content 0.017 g/g dry biomass -Carbon capture rate 42.82 mg C/Ld -Nitrogen capture rate 8.38 mg N/Ld -Phosphorus capture rate 0.91 mg P/Ld -lipid 215.77 mg/g	[99]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Scenedesmus sp. *DDVG I*	Wastewater Treatment Plant (WWTP) in Saint Paul, Minnesota, USA	-Mixotrophic 40.5 µmol/m^2s 12 h:12 h (light: dark) -Heterotrophic in dark condition	27 ± 1 °C	-pH 7.0 -COD 484.8 ± 8.5 mg/L -NH_3-N 40.2 ± 1.1 mg/L -TN 44.9 ± 1.9 mg/L -TP 7.9 ± 0.5 mg/L	10 days	0.3 g/L	-	250 mL Erlenmeyer flask with 100 mL wastewater	-Biomass concentration 3.4 ± 0.13 g/L -Lipid content 22.51% DW -COD removal 75.6 ± 1.1% -NH_3 removal 100% -TN removal 99.8% -TP removal 100%	[44]
Chlorella sorokiniana UTEX 1230	Raw sewage originated from the University Campus (Mytilene, Greece)	Constant light conditions	24 ± 2 °C	-COD (mg/L) 618 ± 300 -NH_4-N (mg/L) 54 ± 14 -TKN (mg/L) 80 ± 22 -TP (mg/L) 4.2 ± 0.30	7 days	-	-	cotton-gauze plugged flasks (total volume 200 mL)	-COD (mg/L) 5 ± 5 -NH_4-N (mg/L) 30 ± 15 -TKN (mg/L) 39 ± 12 -TP (mg/L) 0.9 ± 0.60 -Biomass 169 ± 11 mg/L	[100]
Scenedesmus obliquus	MWW from the effluent of a treatment plant on the central campus of the Universidad Nacional Autónoma de Mexico	-	-	-Nitrate conc. (mg/L) 15.0 ± 4.9 -Orthophosphate conc. (mg/L) 67.5 ± 19.5 -TAN (mg/L) 133.1 ± 13.9 -COD (mg/L) 95 ± 24.7 -TSS (mg/L) 47.9 ± 35.8 -pH 7.5 -Carbohydrates (mg/L) 5.7 ± 3.9	8 days	Initial bio. mass conc.n of 0.2 g/L	Air rate 0.5 VVM	5 L batch PET bioreactors wrapped in aluminium foil	-Biomass 0.98 ± 0.10 g/L -Nitrate removal 60% -TAN removal 53% -Orthophosphate removal 46%	[64]
Chlamydomonas sp. *JSC 4*	Local wastewater treatment plant (WWTP) in Harbin, China	200 µmol/m^2s irradiance	20 °C 30 °C 40 °C	-COD 248.147 mg O_2/L -TN 64.59 mg/L -TP 11.18 mg/L	6 days	80 mg /L	2% CO feeding	1 L batch PBR	-COD removal 100% -TN removal 100% -TP removal 100% -Biomass 3.6 g/L at 30 °C -Lipid production 2 g/L at 30 °C	[45]
Chlorococcum sphacosum	Primary sedimentation tank of Haofeng Wastewater Treatment Co., Ltd. located in Taiyuan, Shanxi Province, China	55 µmol photons/m^2s 12 h:12 h	25 °C	-COD 400 mg/L -Ammonium N 35 mg/L -TP 5 mg/L -pH 7.5	6 days	Initial inoculum 50 mg/L, 100 mg/L, 200 mg/L, 400 mg/L 800 mg/L	-	-	-C removal 94.3–98.6% -N removal 96.8–97.9% -P removal 87–99% -Cell density 1,906,000–40,667,000 cells/mL -Protein content 153.14 mg/g DW at initial inoculum 50 mg/L -Polysaccharides content 45.08 mg/g DW at initial inoculum 50 mg/L	[46]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorella Vulgaris (Z-8 + N)	Effluent of a municipal WWTP in Mashhad City, Iran	-	25.7 °C	-pH 7.7 -BOD5 mg/L 47 -COD mg/L 70 -TSS mg/L 40 -DO mg/L 2 -TKN mg/L 14 -NO_3^- mg/L 50 -NO_2^- mg/L 0.05 -Total phosphate mg/L 6 -Phenols mg/L 0.09 -Ammonia mg/L 0.9 -Cu mg/L 0.2 -Zn mg/L 0.2	Every 12 h for 36 weeks	-	1 L air/min	Erlenmeyer flasks of 1 L	-91% COD removal	[101]
C. vulgaris FACHB-8	Effluent from the anaerobic pool at the Third Sewage Treatment Plant of Xi'an (Xi'an, China)	-	3 temp. regimes: 4 °C 35 °C 35 °C day/4 °C night	-COD (mg/L) 693 ± 8.67 -TN (mg/L) 63.40 ± 2.45 -NH_3-N (mg/L) 43.5 ± 1.2 -TP (mg/L) 7.08 ± 0.09 -pH 7.00 ± 0.08	15 days	7.67 g/L	-	Lab-scale batch 1 L glass PBRs	Best removal rates at 35 °C day/4 °C night, final values: -COD (mg/L) 118.00 ± 3.05 -TN (mg/L) 2.20 ± 0.76 -NH_3-N (mg/L) 0.95 ± 0.54 -TP (mg/L) 0.06 ± 0.07 -Biomass productivity (mg/Ld) 99.21 ± 2.56 -Lipid content 26.4% -Polysaccharide content 36.8% -Protein content 11.6%	[102]
Chlorella sorokiniana pa.91	Real raw MWW influent after primary settling from wastewater treatment plant in Sari, Iran	Different light intensities (1000, 3000, 4000, 5000 and 7000 Lux)	Different temp. (20, 25, 30 and 35 °C)	-sCOD mg/L 211.4 ± 3.0 -N-NO_3^- mg/L 2.01 ± 0.8 -N-NO_2^- mg/L 0.06 ± 0.6 -N-NH_4^+ mg/L 34.1 ± 2.8 -P-PO_4^{3-} mg/L 6.1 ± 0.5 -Ca^{+2} mg/L 56 -Mg^{+2} mg/L 11 -Mn^{+2} mg/L 0.9 -TSS mg/L 73 -Turbidity FAU 102 -pH 7.8 ± 0.3	16 days	-	-	250 mL Erlenmeyer lasks	-Biomass concentration 3.2 g/L at 30 °C and 4000 Lux -Ammonium removal 74% -Nitrate removal 93% -Phosphate removal 83% -COD removal 77%	[103]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Spirulina (Arthrospira) platensis	Aquaculture wastewater from *Pangasianodon hypophthalmus* culture at Aquaculture Division, Central Institute of Fisheries Education, Mumbai, India	19,000–21,730 lx	28.9 °C	-pH 8.26 -DO (mg/L) 3.96 -BOD (mg/L) 68.00 -Alkalinity (mg/L) 178.00 -Turbidity (NTU) 8.10 -TDS (mg/L) 2317.00 -Salinity (mg/L) 1603.70 -Conductivity (µs/cm) 4116.7 -NH_4^+-N (mg/L) 1.00 -NO_3^--N (mg/L) 33.41 -NO_2^--N (mg/L) 1.30 -PO_4^{3-}-P (mg/L) 10.25	27 days	OD_{750} 0.063, 1500 cells/mL	1.25 vvm	Rectangular plastic crates	-Biomass yield 0.36 g/L -SGR 0.21/day -Cell productivity 23.65 mg/L -Doubling time 3.37 days -Phycocyanin 103.29 mg/g (DW) -Carotenoids 1.21 mg/g (DW) -Protein 533.50 mg/g (DW) -NH_4^+-N removed 97.34% -NO_3-N removed 95.90% -NO_2^--N removed 71.93% -PO_4^{3-}-P removed 93.39%	[60]
Tetradesmus (Scenedesmus) obliquus	Discharge outlet of the Tuandao WWTP near the Jiaozhou Bay (Qingdao, China)	60 µmol photons/m^2s 16 h/8 h light/dark cycle	25 °C	-	5 days	2.5×10^5 cells/mL	-	500 mL flasks	-SGR 0.44/d -Biomass 0.217 g/L -Lipid production 0.025 g/L -Lipid content 11.34%	[104]
Microalgal culture	MWW Hyderabad, Telangana, India,	Direct sunlight 12:12 (light:dark)	25–30 °C	-pH 7.2 -TN 101.3 mg/L -P as orthophosphate 5.2 mg/L -COD 492 mg/L -TOC 245.6 mg/L -C:N:P 47:19:1	10 days	-	0.2 L/min	Glass bottles of 500 mL	-TOC removal 89.2% -TN removal 73.1% -TP removal 91% -Lipid content 15%	[47]
Chlorella sorokiniana	Sewage treatment plant located in Almería, Spain	320 µmol photons/m^2s	25 °C 30 °C 35 °C 40 °C 50 °C	-pH 8.1 -Bicarbonate 1574 mg/L -Chlorides 511 mg/L -Carbonates 72 mg/L -Na 238 mg/L -Ammonium 601 mg/L -Ca 124.8 mg/L -K 110 mg/L -Mg 80.0 mg/L -Sulphates 41.1 mg/L -P 15.8 mg/L -B 0.26 mg/L -Zn 0.11 mg/L -Fe 0.04 mg/L -Mn 0.02 mg/L -Nitrates 13 mg/L -Cu 0.08 mg/L	-	0.8 g biomass /L	0.1 v/v min	3 L poly methyl methacrylate bubble column PBRs	-Biomass concentration 1 g/L at 35 °C -Biomass productivity 0.2 g/Ld at 35 °C -Carbohydrates 37% -Protein 32% -Lipid 23% -N removal 88% -P removal 61%	[48]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorella sp.	Real MWW	-	-	-pH 7.8 -TSS 424 ± 341.7 mg/L -COD 497 ± 279.3 mg/L -TC 342 ± 175.7 mg/L -TP 10 ± 5.5 mg/L -TN 64 ± 19.8 mg/L -NH_4^+-N 28 ± 11.4 mg/L	-	-	-	pilot-scale raceway pond located outdoors	-TSS removed 86% -COD removed 70% -TC removed 67% -TP removed 77% -TN removed 65% -NH_4^+-N removed 93%	[49]
Tetradesmus obliquus MACC-677	MWW treatment plant (WWTP) in Třeboň, Czech Republic	Culture circulated only during the day, at night stored in a retention tank (light/dark 12/12 h)	22–27 °C	-BOD 440 mg/L -COD 2100 mg/L -TOC 880 mg/L -Nitrates 1.9 mg/L -N-NO_3 0.43 mg/L -N-NO_2 0.018 mg/L -N-NH_4 160 mg/L -P-PO_4 120 mg/L -TN 290 mg/L -TP 140 mg/L	11 days	0.5 g DW/L	-	-Thin-layer cascade (TLC) -Thin-layer raceway pond (TL-RWP)	-Biomass 3.5 g/L -N-NH_4 removal 98.5% -P-PO_4 removal 89% -TN removal 68% -TP removal 51%	[50]
Chlorella vulgaris UAL-1	MWW treatment plant located in Almería, Spain	12:12 h light:dark	25 °C	-N-NH_4^+ 170.2 mg/L -N-NO_3^- 11.7 mg/L -P-PO_4^{3-} 23.1 mg/L -COD 542 mg/L	9 days	1.0 g/L	-	300 mL bubble column PBR	-Biomass 2 g/L -N-NH_4^+ removal 93.8% -N-NO_3^- removal 73.1% -P-PO_4^{3-} removal 80.5% -COD removal 85.2%	[61]
Chlorella vulgaris	Tilapia wastewater (TW) from ITBoca, a fattening stage tilapia crop	55.5 µmol/m^2s (3000 lx)	22 °C	-NH_4^+ 1.5 mg/L -NO_2^- 7.5 mg/L -NO_3^- 72 mg/L -PO_4^{3-} 10 mg/L -pH 6.58 -COD 23 mg O_2/L	11 days	1×10^6 cells/mL	-	three-litre acrylic tubular photobioreac-tors with an operating volume of 2 L	-Biomass productivity 0.2 g/Ld -NH_4^+ removal 74.6% -NO_3^- removal 94.6% -PO_4^{3-} removal 97.9% -Lipid productivity 40 mg/Ld -Lipid content 39.79%	[65]

Table A1. *Cont.*

Species	Growing Medium	Growth Parameters						Growing System	Results	References
		Light	Temperature	Nutrients Waste Water	Experimental Period	Density	Air			
Chlorella vulgaris *Nannochloropsis salina*	Local MWW treatment plant in Victoria, Australia	180 µmol/m^2s	23 °C	-TDS (g/L) 5.5 -pH 7.7 -DOC (mg/L) 66.4 -COD (mg/L) 164 -TP (mg/L) 13.1 -Ca (mg/L) 68 -K (mg/L) 187 -Sulphate (mg/L) 290 -DO (mg O_2/L) 9.1 TN (mg/L) 43.2 Nitrite-N (mg/L) 1.7 Nitrate-N (mg/L) 37.2 -Ammonia-N (mg/L) 2.6 -Bicarbonate alkalinity (mg/L) 340 -Mg (mg/L) 122	10 days	2×10^6 Cell/mL	No external aeration	Cotton wool-plugged Erlenmeyer flasks maintained in suspension on an orbital shaker	-SGR (d^{-1}) 0.29 -Biomass productivity (mg DCW/L*d) 361 -NO_3-N removal (mg/L*d) 10.5 -NO_2-N removal (mg/L*d) 0.5 -NH_3-N removal (mg/L*d) 1.2 -TN removal (mg/L*d) 11.4 -TP removal 80% -DIC removal (mg/L*d) 35.5 -SGR (d^{-1}) 0.29 -Biomass productivity (mg DCW/L*d) 335 -NO_3-N removal (mg/L*d) 9.4 -NO_2-N removal (mg/L*d) 0.8 -NH_3-N removal (mg/L*d) 1.2 -TN removal (mg/L*d) 10.8 -TP removal 80% DIC removal (mg/L*d) 35	[70]
Chlorella sp.	Open domestic wastewater stream	No illumination	20 °C	-COD 424.24 mg/L -TOC 275.60 mg/L -TN3.278 mg/L -TP 0.04 mg/L -Ammonium 24.4 mg/L	12 days	-	-	500 mL Erlenmeyer flasks	-100% removal rate for COD (424 mg/L) -53% of fats from the dry mass	[105]

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