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Review

Microalgal Systems for Wastewater Treatment: Technological Trends and Challenges towards Waste Recovery

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Abstract: Wastewater (WW) treatment using microalgae has become a growing trend due the economic and environmental benefits of the process. As microalgae need CO_2, nitrogen, and phosphorus to grow, they remove these potential pollutants from wastewaters, making them able to replace energetically expensive treatment steps in conventional WW treatment. Unlike traditional sludge, biomass can be used to produce biofuels, biofertilizers, high value chemicals, and even next-generation growth media for "organically" grown microalgal biomass targeting zero-waste policies and contributing to a more sustainable circular bioeconomy. The main challenge in this technology is the techno-economic feasibility of the system. Alternatives such as the isolation of novel strains, the use of native consortia, and the design of new bioreactors have been studied to overcome this and aid the scale-up of microalgal systems. This review focuses on the treatment of urban, industrial, and agricultural wastewaters by microalgae and their ability to not only remove, but also promote the reuse, of those pollutants. Opportunities and future prospects are discussed, including the upgrading of the produced biomass into valuable compounds, mainly biofuels.

Keywords: agroindustrial wastewater; industrial wastewater; microalgal bioproducts; urban wastewater; strain isolation

1. Introduction

The United Nations estimates that around 2212 km^3 of wastewater (WW) are released annually, approximately 56% of all the freshwater used, of which ~80% is discharged without any prior treatment [1]. On the other hand, despite the investment made by high-income countries in WW treatment, only a very small part of the treated water is reused. As it has been estimated that by 2030 the world will suffer a water deficit of 40%, it is urgent to search for sustainable processes enabling the reuse of wastewater [2]. Among these, microalgal cultivation could be a feasible alternative to existing WW treatment processes. Urban, industrial, and agroindustrial wastewater is usually rich in organic and inorganic compounds, mainly nitrogen, carbon, and phosphorus, which can be used as a nutritional source for microalgal crops [3]. In addition to these nutrients, effluents may contain compounds such as pesticides, heavy metals, and pharmaceuticals, and the ability of microalgae to metabolize these compounds makes their cultivation even more attractive [4–6].

Phosphorus (P), carbon (C), and nitrogen (N) are the main nutrients for microalgae growth. Carbon may be absorbed as CO_2 from the air, industrial exhaust, or soluble carbonates. Nitrogen is an essential nutrient, which is often taken up by microalgae in the form of ammonium due to its greater assimilation simplicity and lower energy consumption [7]; however, in high concentrations, it can be toxic to cells and induce damage to the photosynthetic apparatus [8]. Controlling pH and temperature to limit the concentration of free ammonia or the dilution of wastewater are methods suggested to decrease its toxicity [7,9]. Nevertheless, the dilution should be a strategy to avoid, as it does not make sense to use a scarce source (fresh water) to clean a dirty one. Phosphorus is fundamental for microalgal metabolic processes and is usually found as inorganic phosphate or in organic compounds in effluents. In bioreactors, phosphorus is oxidized to phosphate because of high oxidative conditions [10]. The use of wastewater as a source of nutrients for microalgae cultivation significantly decreases the environmental impact of wastewater regarding eutrophication, smog formation, or acidification of waterbodies [11]. In addition, wastewater treatment costs can be considerably reduced by using microalgae instead of conventional bacterial treatments, because the latter often have high energy demands [12].

The first studies on wastewater treatment using microalgae are from the 1950s [13] and several improvements have been made since then [14–16]. Among the most significant challenges is the choice of strains/consortia that are able to grow robustly in each wastewater. Therefore, resistance to harmful substances present in the effluents is key to enabling the scale-up process and achieving high biomass growth rates concomitant to a high efficiency of water treatment at a short hydraulic retention time. Another challenge is the development of reactors that are able to provide adequate lighting and agitation to increase the capacity and efficacy of the WW treatment. A possible future avenue is improvements in automation of the whole treatment process to decrease labor costs and increase efficiency. Nevertheless, cost assessments are rarely performed, despite the large amount of research at the laboratory and pilot scale. Cost effectiveness may be a limiting factor for the scale-up of microalgae-based WW treatments. However, microalgae have numerous advantages that might be able to offset the capital (CAPEX) and operational (OPEX) expenditures associated with the treatment process [17].

This review aims to give a more general overview, addressing the treatment of different effluents and the use of the microalgal biomass produced in each effluent. Alternative technologies for improving WW treatment by microalgae will be proposed and the main challenges, technological trends, and future prospects in WW treatment and scalability will be tackled. In addition, this review discusses the main strategies that can be used to improve the ability of microalgae to treat different types of effluents, namely: (i) urban WW (secondary and tertiary treatments thereof, including the removal of pharmaceuticals); (ii) agriculture WW (e.g., wastes from farming—aquaculture, poultry, swine, cow, dairy, and food processing plants from which removal of antibiotics or pesticides is often needed); and (iii) industrial wastes such as flue gas (either on their own or in combination with WW treatment). Opportunities for upgrading the value of microalgal biomass are discussed, including nutrient recycling and biofuels production. Lastly, other possibilities such as biofertilizers and/or biostimulants or the extraction of high-value chemicals such as polyunsaturated hydrocarbons, phenols, flavonoids, or carotenoid pigments are reviewed. The constraints resulting from the contamination of biomass with metals, pharmaceuticals, and pesticides, for example, are also addressed.

2. Urban Wastewater Treatment

2.1. Composition of Urban Wastewater and Treatment Alternatives Using Microalgae

Microalgae use light as a source of energy, while producing O_2 (useful for the bacteria) during photosynthesis, and WW works as a culture medium. Simultaneously, nutrients (inorganic N, P, and C) are consumed and removed from the medium [18]. The oxygenated water allows heterotrophic bacteria to biodegrade organic compounds from wastewater, providing inorganic carbon to be used by microalgae (Figure 1) [8]. Untreated urban wastewater (UWW) constitutes a source of pollution that may endanger and disturb the equilibrium of the environment. To minimize these effects, wastewater treatment plants (WWTPs) receive a complex urban catchment of effluents coming from domestic, commercial, industrial areas, and hospitals [19,20]. Conventional wastewater treatment provides satisfactory levels of carbon, nitrogen, and phosphorous removal at the expenses of high-energy consumption and environmental impacts (high CO_2 footprint and nutrient loss) [15].

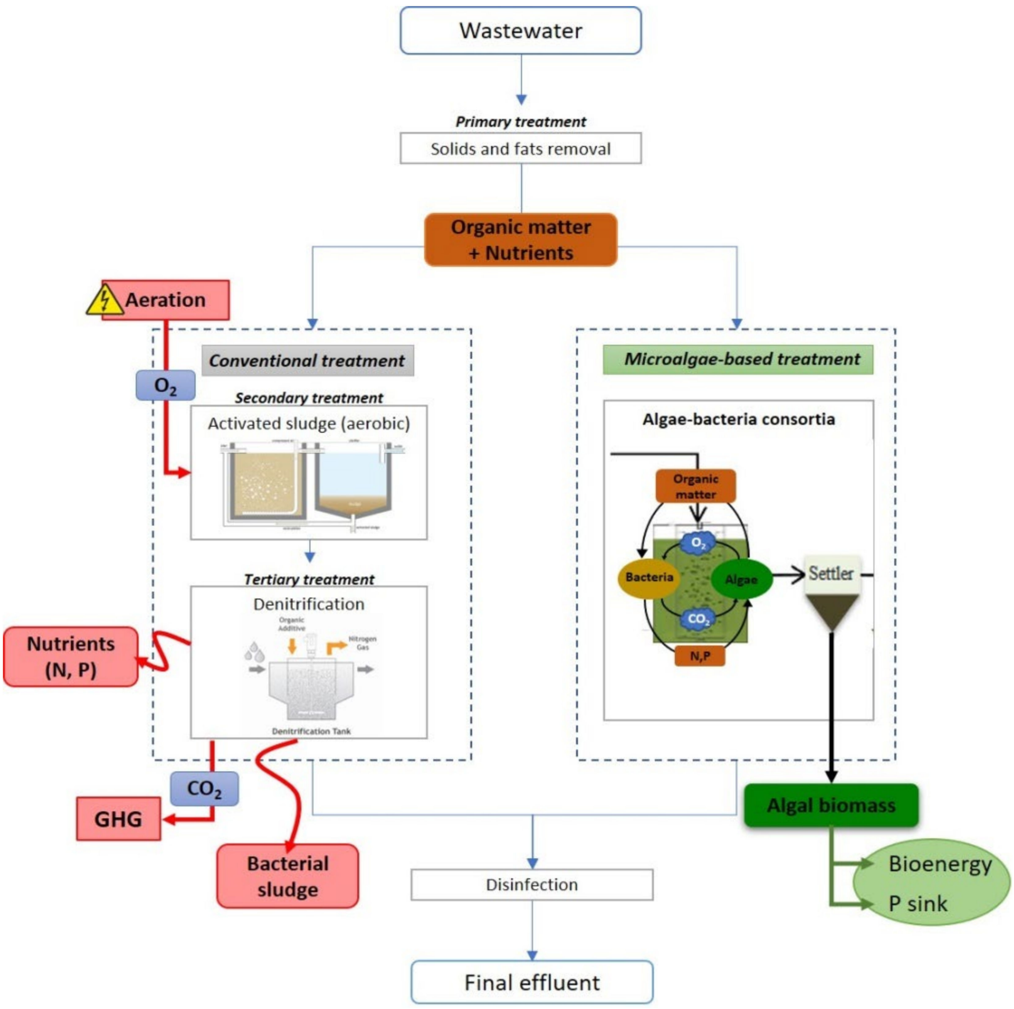

Figure 1. Conventional wastewater treatment versus wastewater treatment-based microalgae [21].

Conventional WW treatment systems consisting of an activated sludge process use large amounts of energy (0.6 kWh/m^3) at a cost of \$0.3/m^3. Microalgae production from WW can produce up to 1 kg of biomass per m^3 of WW by recovering the pollutants/nutrients, at a lower energy consumption and cost [10]. Another advantage of using microalgae is usefulness, as microalgal biomass is rich in several biocompounds, such as carbohydrates, proteins, and lipids, with a very wide range of applications in various industries. Moreover, the ability of microalgae to duplicate within a few days, as well as the ability of some species to be produced throughout the year, makes microalgal WW treatments even more interesting. Although nutrient removal is the focus of microalgae-based WW treatment, the reintroduction of these compounds into the market as products (e.g., biofuels, biofertilizers, biostimulants, high value chemicals) make microalgae part of the circular economy concept [12,14]. In addition, the easy adaptation of microalgae to different climatic conditions allows it to be cultivated near WW sources. To improve treatment efficiency, strategies such as the isolation of local strains from water bodies, as well as use of natural blooms, may be an alternative for obtaining a robust, resistant culture with greater ability to eliminate contaminants and allow water reuse.

A real case of success is AQUALIA, a Spanish company based in Chiclana, which is the largest demonstration facility of the WWT-based microalgae technology worldwide [18]. This facility can treat the WW from the 60,000 inhabitants of Chiclana. The technology reduces the land required to less than 3 m^2/PE (person equivalent) by operating the process at a hydraulic retention time of 2 days; this supports an average biomass production capacity of up to 90 t/ha year, close to the theoretical values for autotrophic growth [18]. This elevated productivity is achieved through the development of "mixotrophic" cultures consisting of microalgae and bacteria, which are capable of efficiently removing contaminants from WW while producing clean water complying with national and European regulations. In the ALL-GAS case study, up to 80% and 90% of total N and P removal efficiency was achieved, respectively, at an energy consumption of 0.2 kWh/m^3. The effluent generated in the ALL-GAS plant complies with the most restrictive limits set by the European directive, with an annual production of biogas enough to run 325,000 km by seven cars and a bus, as well as the production of 40–60 tons of biomass for biofertilizers [18].

In studies by García et al. [22], Gouveia et al. [17], and Mennaa et al. [12], both single species and consortia of different microalgae strains were able to treat urban WW (Table 1). WW treatment technology using microalgae depends on the initial composition of the medium, weather conditions, and light intensity, all of which will determine the composition of the microalgal population during the treatment. In the meantime, the population is continuously modulated in a dynamic way according to external, interspecific, and intraspecific factors that determine the point of balance between the treatment capacity and its efficiency. During an operation period of 176 days, García et al. [22] found shifting dominance between *Chlorella* sp., *Tetradesmus obliquus*, and *Aphanothece* sp. in the same algal-bacterial photobioreactor. In addition, Gouveia et al. [17] reported similar results regarding nutrient removal when using single-strain cultures of *Chlorella vulgaris* or *Tetradesmus obliquus* and when a consortium comprising *Chlorella*, *Chaetophora*, *Tetradesmus*, and *Navicula* was applied. However, biomass productivity was very different in each case. Mennaa et al. [12] and Ferreira et al. [23] reported, however, that *S. obliquus* (*Tetradesmus obliquus*) had the best performance in nutrient removal and biomass productivity.

Treated water should be released into streams following the standard limits for nitrogen, phosphorus, and organic carbon demand (COD) defined by the European Union Directive 91/271/CEE. However, WWTPs are facing new challenges against the so-called priority substances or emerging pollutants, such as biocides, pesticides, polycyclic aromatic hydrocarbons, chlorinated solvents, pharmaceuticals, personal care products, cosmetics, and endocrine disruptors, listed in the 2008/105/EC European Directive for the establishment of environmental quality standards. The removal efficiency of organic chemicals by conventional treatments is better than for pharmaceutical compounds, but the removal efficiency of most pollutants is still insufficient or incomplete and highly variable [19,20].

Blanchard et al. [24] reported 98% and 76% of removal efficiency of PAHs and PCBs, respectively, and Deblonde et al. [19] described removal rates of over 90, 71, and 30–50% for, respectively, phthalates, BPA, and pharmaceuticals by conventional WWTPs. Once disposed of into the environment, pollutants are subjected to physicochemical and biological processes, including filtration, sorption, biodegradation, and chemical transformation (e.g., oxidation, hydrolysis, and demethylation), which can lead to the production of metabolites with greater toxicity than the original compounds [25]. The study by Xie et al. [26] on BPA and tetracycline (TCY) (1, 5 and 10 mg L^{-1}) bioremediation by *Chlamydomonas* sp. Tai-03 reported a 100% removal at all BPA and TCY concentrations and determined that these compounds were removed by the combination of two mechanisms: photolysis and hydrolysis.

The pH of the WW also influences WW biodegradability in microalgae-bacteria systems. Therefore, WW with a pH outside of the optimal range for their treatment in a photobioreactor (7–9 without any pH adjustment) is hardly biodegraded [9,15,27]. The concentration of C, N, and P in WW and the nature of these elements also influences the final algal-bacterial biomass composition [27] and, therefore, its application.

Table 1. Nutrient removal efficiencies from urban wastewater using microalgae.

Species	Final TN or % of TN Removed	Final TP or % of TP Removed	TSS	Final COD or % COD Removal	Ref.
Tetraselmis sp. CTP4	12.2 mg L^{-1}	5.1 mg L^{-1}		45.1 mg L^{-1}	[13]
Natural algal bloom	4 mg L^{-1} at 144 h or >99%	0.05 mg L^{-1} at 144 h			[28]
7 species and microalgal bloom	>87%	>80%			[12]
Tetradesmus sp.	79%	57%		84%	[23]
Microalgae-bacteria consortia	1.2 ± 1.2 mg L^{-1} or >95%		7.4 ± 6.2 mg L^{-1}	85%	[29]
Mixed microalgae culture	Complete removal	Complete removal	1.1 g L^{-1} during 30 days	70% within 8 days	[30]
Three microalgae and consortium	84–98%	95–100%		36–64%	[17]
Mixed microalgal culture	97%		230–240 mg L^{-1}	80%	[31]
C. kessleri and *C. vulgaris*	>95%	>98%			[32]
Tetradesmus actus	94%	66%		77%	[33]
Microalgal inoculum	>80%	>80%		>80%	[34]
Legal limit	15 or 10 mg L^{-1} or 70–80%	2 or 1 mg L^{-1} or 80%	35 or 60 mg L^{-1} or 70%	125 mg L^{-1} or 75%	Council Directive 91/271/CEE

2.2. *Microalgae as an Alternative to the Removal of PPCPs (Pharmaceutical and Personal Care Products) from Urban Effluents*

The increasing consumption of PPCPs is evident. Since it is a widely diverse group, which is composed of thousands of substances developed to produce biological effects, PPCPs are considered persistent pollutants in the environment. At the same time, a synergistic effect is created by their continuous release into the environment, increasing the impact of these pollutants. To avoid hazardous effects on the environment, the removal of PPCPs depends on the effectiveness of chemical and/or biological treatments performed at WWTPs. Conventional WWTPs were not designed to treat emergent pollutants and often fail at this task. Hence, the treatment of urban effluents must begin with an environmental risk assessment, following the monitoring and specific application of new and emerging technologies able to remove persistent toxic organic chemicals.

Urban WW is the main source of PPCPs in the environment. These effluents contain antibiotics, anti-inflammatory and analgesic drugs, β-blockers (cardiovascular drugs), tranquilizers, stimulants, lipid regulators, steroids, hormones, fragrances, skin care products, sunscreen agents, and soaps, among others [35]. Hormones (e.g., estrone and 17β-estradiol), stimulants (e.g., caffeine), antibiotics (e.g., ciprofloxacin, doxycycline, norfloxacin, trimethoprim and sulfamethoxazole), and analgesic and anti-inflammatory drugs (e.g., diclofenac, naproxen, ibuprofen, the antiepileptic drug carbamazepine, and the disinfectant, triclosan), are commonly found in urban waters. Many others are also present, as shown in a study that identified at least 78 different pharmaceuticals in hospital effluents and WWTPs [20].

The presence of antimicrobials in WWTPs promotes the development of bacterial resistance that could be generated via mutation, horizontal gene transfer, and stress factors [36]. When these contaminants are present, the aquatic environment is susceptible to bioaccumulation at several tropic levels of the food web and there are several cases in which the toxicity threshold is surpassed, as shown in Pereira et al. [37]. Maranho et al. [36]

showed that the sea urchin *Paracentrotus lividus* is susceptible to embryotoxicity caused by pharmaceuticals. In the Mediterranean mussel *Mytilus galloprovincialis*, Gonzalez-Rey and Bebianno [38] showed that ibuprofen and the β-blocker propranolol may lead to endocrine disruption and affect cell signaling, respectively. A study using carbamazepine revealed the induction of oxidative stress on bivalves [39]. A sunscreen promoted coral bleaching by viral infection as described by Danovaro et al. [40]. Li et al. [41] analyzed the risks of caffeine and the bioaccumulation in aquatic organisms, confirming the existence of several impacts including altered metabolic activity and neurotoxic effects.

Microalgae and microalgal-bacteria consortia can also be adversely affected by PPCPs including triclosan, clarithromycin, spiramycin, and tetracycline [42]. Under outdoor conditions, DeLorenzo and Fleming [43] showed that only triclosan was toxic to the marine microalga *Dunaliella tertiolecta*, but a mixture of PPCPs could decrease the toxicity threshold. However, several authors have described microalgae as highly resistant to pharmaceuticals. For example, Xiong et al. [5] studied the effect of enrofloxacin, a fluoroquinolone antibiotic, on the freshwater microalgal species *Tetradesmus obliquus*, *Chlorella vulgaris*, and *Chlamydomonas mexicana*, individually and in consortia, revealing that these species could tolerate high concentrations of the drug and that all microalgae were able to recover from the exposure to enrofloxacin. In another study, Guo et al. [39] showed that the lipid-rich microalgae *Chlorella* sp. Cha-01, *Chlamydomonas* sp. Tai-03, and *Mychonastes* sp. YL-02 were highly resistant to cephalosporin antibiotics without displaying signs of toxicity while accumulating the drug. In fact, the sensitivity of some microalgae strains to PPCPs appears to be an exception.

Several procedures have been developed aiming for PPCP removal, such as adsorption with activated carbon, graphene and graphene oxide, UV treatment, irradiation, and ozonation [44]. However, biological processes such as microalgae-based technologies have received increasing attention by the scientific community, particularly those using *Tetradesmus* sp. and *Chlorella* sp., which are considered pollution-tolerant microalgae [24,45]. Microalgae-based treatment is dependent on the physicochemical properties of each compound and, removal efficiencies are quite variable, ranging from 0 to 100% (Table 2) [46–58]. The removal of PPCPs depends on physical and chemical processes that occur in microalgal treatment systems, such as photolysis, hydrolysis, oxidation/reduction, and the mechanisms used by microalgae themselves include adsorption, bioaccumulation, biodegradation, and biotransformation, involving enzymatic mechanisms such as hydroxylation, glycosylation, and epoxidation, among others (Figure 2) [42,52]. The removal efficiency of contaminants depends additionally on abiotic factors such as redox potential [53], pH, temperature [54], and light [46]. Other biotic factors might include cell size and the composition and structure of microalgal cell coverings (e.g., cell wall, theca, testa, glycocalyx, or scales, and the absence or presence of mucilage, exopolysaccharides, and other extracellular polymeric substances), which can affect the binding of the pollutant to the cell [39].

Table 2. Microalgae used for pharmaceuticals and personal care products removal from WWs.

Microalgae	Compound	Removal	Ref.
Nannochloris sp.	Trimethoprim Sulfamethoxazole Triclosan	0% after 14 days 32% after 14 days 100% after 7 days	[58]
Chlorella sorokiniana CCAP211/8K	Diclofenac, ibuprofen, paracetamol, metoprolol, carbamazepine and trimethoprim	40–60%, 99%, 99%, 100%, 30% and 40–60%, respectively	[48]
Chlorella sorokiniana CCAP211/8K, *Chlorella vulgaris* SAG 221-12 and *Tetradesmus obliquus* SAG 276-1	Diclofenac	29%, 21% and 79%, respectively	[50]
Chlorella sorokiniana CCAP211/8K	Paracetamol and salicylic acid	41% and 93%, respectively	[50]

Table 2. *Cont.*

Microalgae	Compound	Removal	Ref.
Dictyosphaerium sp. (Most frequent)	9, 14, 11 and 18 pharmaceuticals	90%, 50–90%, 10–50% and 10%, respectively	[46]
Selenastrum capricornutum	Ethinylestradiol	92% yield conversion	[55]
Chlorella sp. Cha-01, *Chlamydomonas* sp. Tai-03 and *Mychonastes* sp. YL-02	Cephalosporin	74%, 65% and 60% at 24 h, respectively	[39]
Native microalgae	26 emergent organic contaminants	None to 99%	[51]
Scenedesmus obliquus and *Chlorella pyrenoidosa* FACHB-9	Progesterone and norgestrel	95% within 5 days for both microalgae and almost complete for *S. obliquus*, but nearly 40% with both	[59]
Microalgae consortium and secondary activated sludge	Ibuprofen, naproxen, salicylic acid, triclosan and propylparaben	94%, 52%, 98%, 100% and 100%, respectively	[47]
Chlamydomonas sp. Tai-03	bisphenol A and tetracycline sulfamethoxazole	Complete removal 20%	[26]
Scenedesmus obliquus	Sulfamethazine and sulfamethoxazole	62% and 46%, respectively	[56]
T. obliquus HM103383, *C. mexicana* GU732420, *C. vulgaris* GU732416, *O. multisporus* GU732424, *M. resseri* FR751189 and their consortium	Enrofloxacin	Ranged between 18–26%	[5]
C. vulgaris	Metronidazole	100%	[60]
Chlamydomonas mexicana FR751193 and *Tetradesmus obliquus* HM103383	Carbamazepine	37% and 30%, respectively, after 10 days	[52]

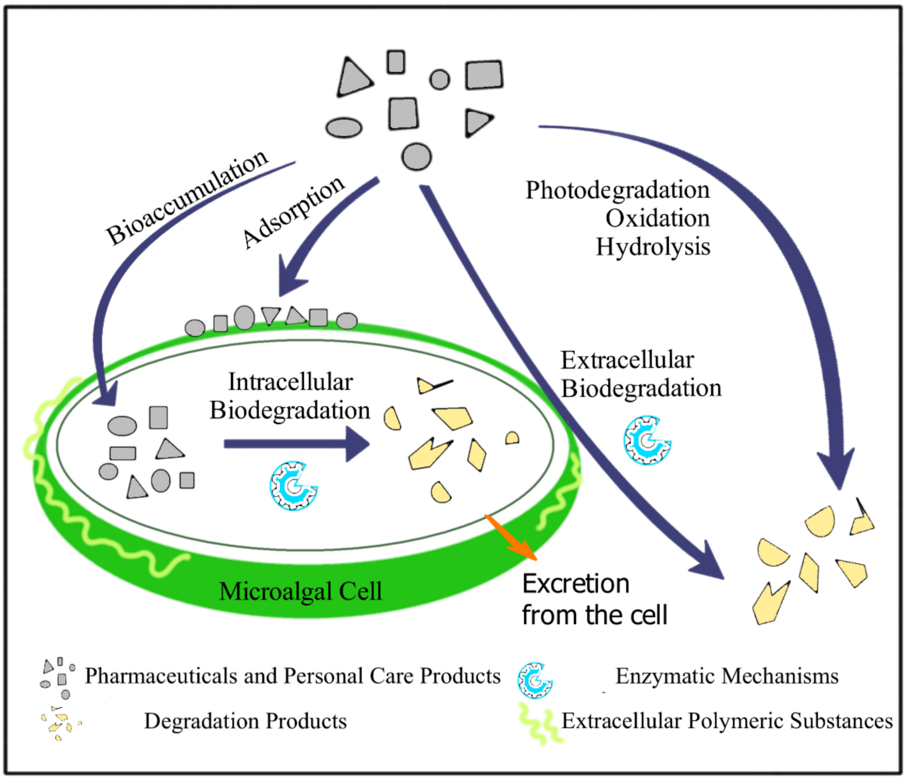

Figure 2. Processes involved in the removal of PPCPs using microalgae-based technologies.

The removal of cephalosporin by microalgae, for example, is mediated by processes such as hydrolysis, photolysis, and biosorption [40]. However, Della Greca et al. [55]

demonstrated the ability of four microalgae species to biotransform the estrogenic contaminant ethinylestradiol by glycosylation and hydroxylation. Nevertheless, it is important to note that co-metabolic activity for biodegradation and/or biotransformation depends on the microbial community. *T. obliquus* was able to remove sulfamethazine and sulfamethoxazole by means of enzymatic reactions that included hydroxylation, methylation, nitrosation, and deamination [56]. Xiong et al. [57] reported the ability of *Chlorella pyrenoidosa* to adsorb sulfamethoxazole and then biotransform it into other metabolites. The proposed mechanism involved the breakdown of the contaminants side chain, coupled with oxidation and hydroxylation reactions of the amine group on the benzene ring, and pterin-related conjugation. Further studies are needed to investigate microalgae-based removal mechanisms but, overall, these examples have shown that microalgae could aid in the process of PPCPs removal as an alternative or in combination with conventional WWTPs.

Pharmaceuticals are widely used chemicals that even in trace amounts are of concern, as they are made to have biological effects [57]. By the literature presented in this section, it is possible to confirm that microalgae are a sustainable and viable alternative for the removal of PPCPs from wastewater, either as an only treatment or after conventional treatments, for the safe release of the treated water to the environment. The mechanisms of removal will determine the possible applications of the produced biomass due to the possibility of storage of PPCPs inside the cells, which might restrict the application of the produced biomass as biofertilizers or as animal feed.

3. Agroindustrial and Industrial Wastewater Treatment

3.1. Harnessing Pollutants from Agroindustry

The type of agriculture WW and its characteristics and pollutant concentrations determine the efficiency of microalgae for WWT, which is also dependent on product type, productivity, operating conditions, and location. The initial C:N:P ratio of the WW is often correlated with its biodegradability in the absence of inhibitory or recalcitrant compounds [15], the optimum biodegradability ratio being 100:18:2 (*g*/*g*/*g*) [9]. $N\text{-}NH_4^+$ at concentrations higher than 100 mg $N\text{-}NH_4^+$ L^{-1} and pH > 8 can inhibit photosynthetic activity in some species because of NH_3 toxicity [9], and microalgae inhibition increases at high pH values. Therefore, effluents with high NH_4^+ concentrations such as livestock wastewaters (~600–3000 mg $N\text{-}NH_4^+$ L^{-1}), centrates (~400–800 mg $N\text{-}NH_4^+$ L^{-1}), or anaerobically digested agroindustrial effluents (~600–800 mg $N\text{-}NH_4^+$ L^{-1}) need to be previously diluted or provided at low loading rates to avoid microalgae inhibition [27]. Heavy metals can also be present in agroindustry WW, which inhibit bacterial growth and photosynthesis and even generate morphological modifications in microalgae cell walls at very low concentrations [61]. Toxic organic pollutants such as salicylate, phenol, phenanthrene, and hydrocarbons also decrease the activity of microalgae and bacteria.

Dairy, olive oil, winery and brewery industries, and animal manure produce effluents rich in nitrogen and phosphorus [6,8,9,14,25,27,61,62] that may be applied as fertilizer onto crop land or used in biostimulant and/or biofuel production. However, excess nitrates, phosphates, insecticides, herbicides, and fungicides can accumulate in the soil, causing environmental damage [63]. The main concern in agroindustry WW is its high content of organic matter, which can be removed through aerobic and anaerobic digestion.

Different authors have studied the use of microalgae for the removal of these nutrients, with or without previous removal of organic carbon. Hernández et al. [64] studied the use of microalgae for the treatment of two different agroindustry wastewaters; these authors observed that the high availability of phosphorus in treated pig effluent allowed higher biomass production compared to effluents of the potato industry, at 26.3 and 18.8 mg L^{-1} day^{-1}, respectively. Wang et al. [65] demonstrated that high initial COD values (750 and 1000 mg L^{-1}) did not compromise the growth of *Chlorella pyrenoidosa*, yielding high biomass productivity and efficient removal of organic matter. In fact, this microalga is known for its capacity to grow in mixotrophic mode, enabling its growth in pig wastewater, which is rich in organic carbon and inorganic nutrients (Table 3).

Table 3. Agroindustrial wastewater treatment using microalgae. COD: Chemical Oxygen Demand, TS: Total Solids, TN: Total Nitrogen, NH_3-N: ammoniacal nitrogen, NH_4-N: ammonium, NO_3: nitrate, TP: Total Phosphorus, PO_4-P: phosphate.

Type of WW	Species	t (Day)	COD (mg L^{-1})	TS (mg L^{-1})	TN (mg L^{-1})	N-NH_3/ N-NH_4^+ (mg L^{-1})	NO_3 (mg L^{-1})	TP (mg L^{-1})	PO_4-P (mg L^{-1})	COD Removed (%)	N-NH_3/ N-NH_4^+ Removed (%)	Final TN or % TN Removed	TP Removed (%)	Biomass Productivity	Ref.
Piggery	*C. reinhardtii*	6	1000 ± 19	652 ± 20	80 ± 1	20 ± 1		22 ± 1		20–42	70–90	32.3–52.4%	78–93	8.8 × 10^6 cells mL^{-1}	[66]
Piggery	*C. pyrenoidosa*	10	11,000	7000	980	1388		158		36.5–57.6	91.2–95.1	54.7–74.6%	31–77.7	100–300 (mg L^{-1})	[65]
Piggery	*C. sorokiniana*		616	3319	32.9	12.3	53.8	50.1		62.3	82.7	0.08 (mg TKN $g^{-1}day^{-1}$)		26.3 (mg $L^{-1}day^{-1}$)	[64]
Swine	*L. maxima*		577.83	1.51	4.67	83.4			3.97		75		53		[67]
Poultry and swine slaughter-house	*Phormidium* sp.		4100 ± 874	3.8 ± 2.7	128.5 ± 12.1				2.84 ± 0.2	97.6		85.5%		0.34 (kg sludge $kgCOD^{-1}$)	[68]
Winery	*Leptolyngbya, Limnothrix*	17	4675	89.21 ±0.1	25.12 ± 9.8		11.03 ± 0.1		5.8 ± 0.3	95–97.4		80–87.7%		79.56–98.9 (mg $L^{-1}day^{-1}$)	[69]
Potato processing	*C. sorokiniana*		1536	1603	33.7	12.1	n.d.	4.2		84.8	>95	0.25 (mg TKN $g^{-1}day^{-1}$)		18.8 (mg $L^{-1}day^{-1}$)	[64]
Palm oil mill effluent	*C. sorokiniana*	15	27,700		1100	172		180		45.05–47.09		54.23–62.07%	29.20–30.77	1.86–2.12 (g L^{-1})	[70]
Sago	*A. platensis*	14	1340 ± 520	690 ± 450		2.87 ± 0.48	40.0 ± 1.33		21.0 ± 4.21	98	99.9			610 (mg L^{-1})	[71]

Agroindustrial wastewaters are an important source of ammonia for the environment. Cañizares and Dominguez [67] studied the use of Limnospira maxima to treat swine wastewater at different concentrations and obtained removals of 75% and 53% for $N\text{-}NH_4$ and total phosphorus, respectively, with a dilution of 50% of the wastewater, achieving high biomass production. Other authors found similar results when treating palm oil mill effluent (POME). The maximum biomass production (2.02 g L^{-1}) was only possible in 20% (v/v) of POME when using *Chlorella sorokiniana* CY-1, with growth being inversely proportional to the concentration of POME [70]. *Tetradesmus dimorphus* was also effective in decreasing the concentrations of ammonia, phosphorus, and COD in anaerobically digested POME by 99.5, 98.8, and 86%, respectively [72]. In comparison, *Chlamydomonas* sp. UKM6 removed between 8.59 and 29.13% COD in undiluted POME [73]. The low removal rates result from a decreased light penetration in the WW due to the large amounts of suspended solids, thus interfering with light penetration and restricting microalgal growth [65,69].

Posadas et al. [9] studied the behavior of a microalgal consortium (*Phormidium*, *Oocystis*, and *Microspora*) in the treatment of effluents from potato and fish processing, coffee manufacturing, animal feed production, and yeast production. Effluents were tested at different dilution rates and it was concluded that high $N\text{-}NH_4^+$ concentrations, high pH values, and biodegradable organic carbon were the limiting factors for efficient WW treatment in most of the effluents tested. These authors stated the need for dilution of high $N\text{-}NH_4^+$ concentrations, pH control, and for an external carbon (CO_2) source for the efficient treatment of agroindustrial WW.

The natural presence of other microorganisms in WW treatment systems has been explored concurrently with the use of microalgae. Tsolcha et al. [69] studied the ability of a consortium of cyanobacteria and heterotrophic bacteria to remove nutrients from viniculture WW with simultaneous biomass and lipid production, yielding a maximum biomass production of 230.73 mg L^{-1} day^{-1} for mixed winery and raisin WW and 21% lipid for winery WW. The uncommonly high sedimentation rate observed for this biomass can be explained by the capacity of microalgae and bacteria to form aggregates that deposit faster, contributing to a more affordable harvest [64]. These authors also studied the synergism between microalgae (*C. sorokiniana*) and aerobic bacteria for the treatment of WW from the potato industry (PIW) and pig manure (PMW), concluding that this consortium was able to remove 82.7% of ammonium from PMW and more than 95% from PIW. Recently, Dias et al. [74] also reported the advantages of yeast and microalgal mixed cultures over pure ones, due to the enhancement of biomass and lipid productivities, cell oil content, and treatment efficiency. Therefore, synergism between microalgae and microorganisms like bacteria and yeast can bring ample benefits to the treatment of agroindustrial WW.

Overall, the studies previously mentioned confirm that there are several microalgae that can be used in agroindustrial WW treatment, although some, such as chlorophytes, are more prominent, probably because they can be found in a wider geographical distribution and are composed of a wider number of different species. Many studies confirmed the efficiency of *Chlorella* microalgae in removing nitrogen and phosphorus from different sources of agroindustrial WW [64,65,70]. *Tetradesmus* spp. appear to be equally capable of removing total nitrogen and phosphorus from WW, while the removal rate of phosphorus by *Chlamydomonas reinhardtii* and *Chlorella kessleri* are much lower (Table 3) [63]. Agroindustrial WW treatment by microalgae is also an opportunity to apply a circular economy system, as the produced biomass can return to the industry as a biofertilizer for the crops. Circular economy in WW treatment by microalgae will be further discussed in the following sections.

3.2. Removing Hormones and Pesticides from Agroindustry WW

The animal agroindustry is one of the largest WW producing industries. In addition to excess nutrients, this WW contains other hazardous compounds like veterinary pharmaceuticals and hormones. Soluble metal salts and antibiotics are added to animal feed to promote animal growth or for the treatment of diseases; hence they are present in high amounts in animal wastes [6]. Zhang et al. [75] found daily estrogen excretion values of 145.23–2394.27 (μg day^{-1} per animal) for cattle, 0.43–7.59 (μg day^{-1} per animal) for swine, and 0.66–12.78 (μg day^{-1} per animal) for chicken, which may vary with the age, diet, and health of the animal.

Regarding antibiotics, Massé et al. [76] stated that the amount of oxytetracycline present in swine manure can reach 354 mg L^{-1}, with only 55 to 75% being removed through anaerobic digestion. Concentrations of 98 and 764.4 mg L^{-1} of tetracycline and chlortetracycline, respectively, were also reported in swine manure [76]. These compounds are hazardous and remain stably bound to soluble organic compounds during manure storage, becoming free when manure is applied to agricultural fields as fertilizer and contaminating water and soil [76]. Similarly, parasiticides and pesticides used in agribusinesses, agriculture, and viticulture are also of concern to human health and to the environment. The ability of microalgae to remove toxic pollutants such as hormones, pesticides, and heavy metals has been shown in several studies (Table 4). These studies have demonstrated that the removal efficiency of such toxic elements by microalgae is influenced by factors such as contaminant concentration, microalgal species, pH, and the nutrients present in the solution [8].

Table 4. Removal rate of pesticides, hormones, and heavy metals by microalgae.

Microalgae	Compound	Removal (%)	Ref.
	Pesticides		
Tetradesmus obliquus	Dimethomorph	24	[77]
Tetradesmus quadricauda		15	
Tetradesmus obliquus	Isoproturon	54	[77]
Tetradesmus quadricauda		58	
Tetradesmus obliquus	Pyrimethanil	7	[77]
Tetradesmus quadricauda		10	
	Hormones	%	
Tetradesmusdimorphus	17a-estradiol	85	[75]
Chlamydomonas reinhardtii	17b-estradiol	100	[78]
Selenastrum capricornutum		88–100	
Tetradesmusdimorphus	17b-estradiol	95	[75]
Tetradesmusdimorphus	Estriol	95	[75]
Tetradesmus obliquus	Progesterone	>95	[59]
Chlorella pyrenoidisa		>95	

Kurade et al. [4] showed that the microalga *Chlorella vulgaris* was able to metabolize the pesticide diazinon into the less/non-toxic compounds 2-isopropyl-6-methyl-4-pyrimidinol (IMP) and diethyl thiophosphate (DETP), unlike physicochemical treatments that led to the formation of diazoxon, a metabolite ten times more toxic than diazinon. The best removal rates were found for an initial diazinon concentration of 0.5 and 20 mg L^{-1}, showing 100% and 93.31% removal rates, respectively, after 12 days of cultivation, and biodegradation appeared to be the main removal mechanism, as degradation metabolites could be observed in HPLC and GC-MS analyzes. Mehta and Gaur [79] showed that for an initial concentration of 2.5 mg L^{-1}, Ni and Cu sorption by *Chlorella vulgaris* were 70 and 80%, respectively. Conversely, when their concentration was increased to 10 mg L^{-1}, Ni and Cu sorption were only 37 and 42% (Table 4).

According to Peng et al. [59] and Zhang et al. [75], the hormones 17α-estradiol, 17β-estradiol, and progesterone were eliminated from WW via biodegradation. The degradation products were unknown, but they were most probably produced by a microalgae–bacteria consortium. Even though the published data on the application of microalgae to remove toxic pollutants present in WW is scarce, several studies have shown the efficient removal of these compounds, with potential applications in agroindustry.

3.3. Industrial Wastewater Treatment

Industrial activities play a central role in our society, as well as in economic growth. However, these activities can have negative impacts on our environment and well-being, as they release chemicals contaminating air, soil, surface and groundwater, and living organisms [80]. The physicochemical characteristics of such contaminants, namely their absorption/adsorption, dissolution/precipitation, degradation, bioaccumulation, and volatilization promote their persistence and toxicity. Industries have been increasing the risk and frequency of environmental hazards, loss of life, and injury due to their rapid development. The most relevant stakeholders are the petroleum industry and the manufacturers of pharmaceuticals, agrochemicals, hazardous chemicals, plastics, and electronics. All these products are detrimental when they reach critical concentrations in the environment. Effluents rich in inorganic chemicals are particularly difficult to dispose of and can be classified as hazardous and non-hazardous; some are hazardous to human health even at low concentrations, for example, arsenic, lead, mercury, and vinyl chloride. In addition, some toxic metals such as cadmium are considered carcinogens, mutagens, and endocrine disruptors; copper causes brain and kidney damage, and mercury leads to autoimmune diseases, among other medical conditions [8].

Contaminants such as heavy metals need to be removed from water, as they are harmful to the environment and human health. Heavy metals are a major concern, because they are not biodegradable and are toxic and persistent in nature. Conventional techniques such as electrochemical treatments, adsorption, and membrane filtration are often very cost-effective [81–88]. In contrast, microalgae are able to efficiently remove heavy metals from aquatic systems in an environmentally friendly way (Table 5) [81–89]. Markou et al. [6] reported the presence of extremely high concentrations of heavy metals in swine manure, reaching 8800 μg L^{-1} of zinc, 2700 μg L^{-1} of manganese, 2100 μg L^{-1} of copper, 9 μg L^{-1} of cadmium; and up to 810 μg L^{-1} of nickel, 1740 μg L^{-1} of lead, and 160 μg L^{-1} of cadmium in wine and vinasse industry WW. Some of these metals such as Cu, Cr, Co, Mn, Ni, Se, Mo, and Zn, are essential for animal physiological activities in small amounts, but can be toxic when present in high quantities [6]. Markou et al. [90] analyzed the biosorption kinetics of Cu and Ni metals by *Arthrospira platensis* and found that the largest removal of metals was obtained between 15 and 30 min after adding the dried biomass, reaching an equilibrium in between 30 and 60 min. Tests with living microalgae showed that biosorption could last for up to 48 h, probably due to variations in metabolic process.

The first stage of heavy metal removal by microalgae is physical adsorption onto the cell covering and the second stage is biosorption, which is slower since it depends on transport and intracellular chelation [87]. In addition to absorption, microalgae are able to metabolize heavy metals. For example, *Nannochloropsis oculata* metabolized approximately 89.29 ± 1.92% of the copper in the culture and only 10.70 ± 1.92% was adsorbed by this microalga [81]. Other studies have shown that increasing concentrations of contaminants such as copper could cause a decrease in cell number and a variation in growth rate and chlorophyll A content [81]. Arsenic has been recognized as a major contaminant of surface and groundwater. Arsenic is industrially used for a variety of purposes, from agriculture to medicine, and can easily be leaked to aquatic systems [83]. Recent studies on microalgae species capable of removing arsenic, such as *Anabaena* sp., showed removal rates of 78% within 10 d. *C. vulgaris* bioremediated wastewater with molybdenum and manganese with an efficiency exceeding 99% (Table 5) [85,91].

Table 5. Toxic metals removal from wastewater by microalgae.

Microalgae	Compound	Concentration	Removal (%)	Time	Ref.
Anabaena sp.	Arsenic	1.0 g L^{-1}	78.0	10 days	[83]
Botryocossuss sp.	Chromium (VI)	5.0 mg L^{-1}	94.2	7 days	[84]
C. vulgaris	Arsenic	12.0 mg L^{-1}	14.9	3 days	[92]

Table 5. *Cont.*

Microalgae	Compound	Concentration	Removal (%)	Time	Ref.
C. vulgaris	Molybdenum	0.5 mg L^{-1}	80.3	3 days	[91]
C. vulgaris	Copper	0.5 mg L^{-1}	55.0	3 days	[91]
C. vulgaris	Manganese	3.0 mg L^{-1}	99.4	3 h	[85]
Chlorophyceae spp.	Zinc	3.0 mg L^{-1}	91.9	3 h	[85]
Chlorophyceae spp.	Copper	3.0 mg L^{-1}	88.0	10 min	[85]
Dunaliella salina	Chromium	5.0 mg L^{-1}	66.4	120 h	[86]
N. oculata	Copper	0.25 mM 1.6 g L^{-1}	99.9	21 days	[81]
Tetradesmus almeriensis	Arsenic	12.0 mg L^{-1}	40.7	3 h	[85]
Tetradesmus almeriensis	Boron	60.0 mg L^{-1}	38.6	10 min	[85]
T. marina AC16-MESO	Copper	5.0 mg L^{-1}	90.0	3 days	[87]
T. marina AC16-MESO	Iron	5.0 mg L^{-1}	100.0	3 days	[87]
T. marina AC16-MESO	Manganese	5.0 mg L^{-1}	23.4	3 days	[87]

4. Upgrading the Value of Microalgal Biomass

The use of microalgae species for WW treatment tackles several problems, such as the removal of excess nutrients in wastewater and the reduction of energy demand, GHG emissions, and production costs, and the consequent production of high-value metabolites can be applied to different industries. However, before application, it is important to test the obtained biomass, due to the ability of the cells to adsorb and absorb heavy metals and other contaminants. These tests serve as an indicator as to whether these compounds are bioaccumulating and, consequently, cause harm to living organisms. It is also important to test different methods of extracting target-metabolites to deliver a safe product, either to the handler and to the environment. These products include high-value metabolites, biofuels, biofertilizers, plant biostimulants, carotenoids, and antioxidants.

One of the main applications for the biomass produced in wastewater is the production of biofuels, as nutrient recycling offsets one of the biggest drawbacks of microalgae production: the costs of nutritional sources for microalgal growth. Other applications include de production of biofertilizers and biostimulants and high-value chemicals (Figure 3).

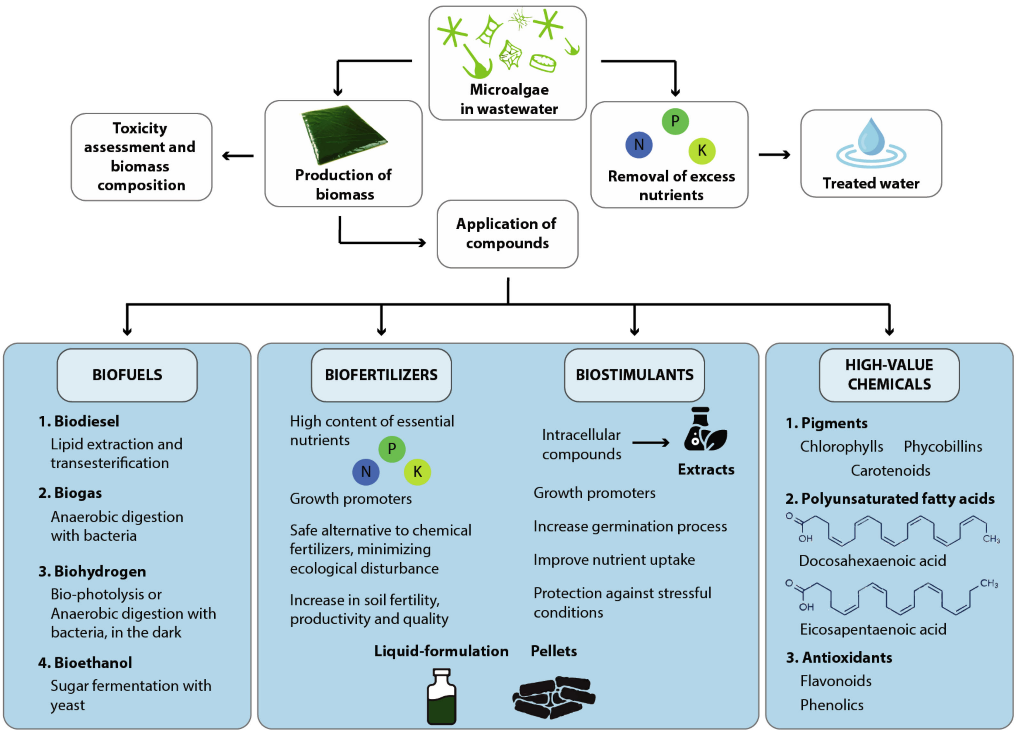

Figure 3. Bioproducts obtained from microalgal biomass produced in wastewater treatment.

4.1. Biofuels

Because of the rise in human population, energy demand is also increasing, as well as concerns about the continuity of fossil fuels, especially in countries with limited access to them. Renewable biofuels have gained much attention, mainly because they are considered the most probable source of energy to replace fossil transportation fuels. Therefore, biofuel production has already been enforced in several countries around the world, showing high potential to improve the sustainability of the energy sector [93].

First (1 GBs) and second-generation biofuels (2 GBs) do not represent viable options, as 1 GBs are primarily obtained from the use of crops exclusively grown for fuel production that compete with food production in terms of arable land and freshwater needs, and 2 GBs from plant scraps that use unsustainable production methods [94]. Instead, several studies have shown that third-generation biofuels (3 GBs) seem to overcome the main challenges faced by 1 GBs and 2 GBs [95]. 3 GBs are mainly produced from microalgae [10], which can be grown on non-arable land using non-potable water, avoiding competition with the agriculture industry [96]. Different biofuels (e.g., biodiesel, bioethanol, biogas, biohydrogen, and biochar) [94–112] can be obtained from microalgae due to their high and unique potential.

From this, interest has been raised in combining the treatment of WW with the production of bioenergy, where microalgae can recover nutrients and incorporate them in biomass, allowing a decrease in biofuel-based microalgae production costs while treating water for reuse. The production of biofuels is not affected by the presence of contaminants in the biomass, but the biomass applicability should be evaluated by analysis of the biomass composition. Several studies have already pointed out that, just by using WW as a source of nutrients, it is possible to achieve a cost-competitive biofuel (Table 6).

Table 6. Biofuels produced in wastewater cultured microalgae.

Microalgae	Wastewater	Biofuel	Ref.
Microalgae consortium	Dairy	Biodiesel	[107]
Chlorella vulgaris	Textile	Biodiesel	[108]
Chlorella sp.	Urban	Biogas	[109]
Chlorella vulgaris, *Tetradesmus obliquus* and *Chlamydomonas reinhardtii*	Piggery	Biogas	[110]
Chlamydomonas reinhardtii UTEX 2243 and *Chlorella sorokiniana UTEX 2714*	Acetate rich wastewater	Biohydrogen	[111]
Chlorella vulgaris, *Tetradesmus obliquus*, *Microalgae consortia*	Urban	Biohydrogen	[112]
Wild yeast and microalgae consortium	Municipal	Bioethanol	[97]
Nannochloropsis oculata and *Tetraselmis suecica*	Municipal	Bioethanol	[95]
Tetradesmus obliquus	Brewery	Bio-oil, BiocharBiogas	[98]

The main interest in using microalgae to produce biodiesel is the high lipid content found in several species (e.g., [93,99]), which can be induced by stresses [94,100–102] and coupled with their high productivity per land surface [10]. However, it is necessary to find ways to reduce the overall cost of the biomass to produce low-value commodities like biodiesel [10]. Biodiesel-based microalgae is obtained through the process of transesterification after lipid extraction, producing fatty acid methyl esters [95], or directly from the biomass (in-situ transesterification) [17].

Some species used to produce biodiesel include *Tetradesmus obliquus* with 61.3% [101], *Chlorella protothecoides* with 55.2% [103], *Neochloris oleoabundans* with 52% [102], and *Nannochloropsis salina* with 37.5% of lipid content [95]. Other microalgae species produce high amounts of hydrocarbons that can be converted into biodiesel, kerosene, and gasoline. In *Botryococcus braunii*, for example, hydrocarbons represent up to 70% of the biomass dry weight [104] and, because they are exported from the cells, the extraction process can be simplified. Additionally, *Botryococcus* hydrocarbons do not need to be esterified. The lipid profile of the selected strain is also important, as microalgae tend to have unsaturated lipid profiles, leading to biodiesel with low oxidative stabilities [99,105]. Hence, strains with low unsaturated profiles are preferable for biodiesel production for reducing down-processing costs. The application of the *Botryococcus* species, however, are limited due to its slow growth and tendency to agglomerate and suffer bacterial contamination due to the secretion of polysaccharides into the culture medium, most of which are antagonistic to its growth [113].

Chandra et al. [107] evaluated the potential for biodiesel production by a microalgal consortium composed of *Mychonastes homosphaera* (formerly known as *Chlorella minutissima*), *Desmodesmus abundans*, *Nostoc muscorum*, and *Arthrospira* grown in a medium containing 70% dairy wastewater supplemented with 10 g L^{-1} of glucose. A biomass concentration of 5.75 g L^{-1} with 20% lipids was obtained, which had a fatty acid composition suitable for biodiesel production, given by the higher abundance of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0s), linoleic acid (C18:2), and linolenic acid (C18:3). Fazal et al. [108] also evaluated biodiesel production from microalgae biomass produced in wastewater, in this case, textile WW, using the microalgae Chlorella vulgaris. The fatty acids found in higher amounts were palmitic acid (C16:0) and linolenoic acid (C18:3), which are suitable for biodiesel production.

Biogas can be produced from microalgal biomass through anaerobic digestion coupled with methanogenic bacteria, a process starting with the hydrolysis of the cell walls to increase biogas yield [114]. Even though there are some concerns about the low C:N ratio typical of most microalgae, there are some alternatives to overcome this constraint, such as the employment of co-fermentation using carbon-rich substrates or nitrogen limitation during algae growth. *Tetradesmus obliquus* biomass can be used to produce biogas with a yield of 287 mL_{biogas} gVS^{-1}, and *Chlamydomonas reinhardtii* with a production of 587 mL_{biogas} gVS^{-1} [106].

Vargas-Estrada et al. [109] studied the integration of microalgae (*Chlorella* sp.) into urban wastewater treatment as a tertiary treatment to recover nutrients and for further energy recovery as biogas. Using non-diluted wastewater, they obtained a biogas production of 204.47 mL/g. According to these authors, the high biogas production was due to the increase in lipid accumulation. Molinuevo-Salces et al. [110] reported the production of biogas from a microalgae consortium cultured on piggery wastewater, reaching a methane production of 106 to 171 mL_{CH4}/g in batch and semi-continuous cultures, respectively. They observed that biomass grown under favorable conditions resulted in higher methane yields.

Biohydrogen can be produced by microalgae or cyanobacteria either by biophotolysis [115,116] or by using microalgal biomass as a substrate for anaerobic bacteria in the dark [117–120], either using *Enterobacter aerogenes* and *Clostridium butyricum* [121]. There are reports of *Chlamydomonas reinhardtii* biomass being hydrolyzed to produce 37.1 mmol $_{H2}$ L^{-1} [122], while *Nannochloropsis* sp. yielded 60.6 mL g^{-1} [123], *Tetradesmus obliquus*, 56.8 mL H_2 g^{-1} [112], and *Tetradesmus obliquus* grown in brewery WW, 67.1 mL $_H$ $g_{SV}{}^{-1}$ [14].

Batista et al. [112] reported the production of biohydrogen through dark fermentation of a microalgae consortium of *Chlorella vulgaris* and *Tetradesmus obliquus* obtained from urban wastewater treatment. The highest H_2 production was obtained using *S. obliquus* (56.8 mL H_2 g^{-1}); a similar value was obtained with the biomass of this microalga grown in synthetic media. Hwang and Lee [111] studied the feasibility of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* grown in acetate-rich wastewater for hydrogen production through photolysis at different light intensities. The authors demonstrated that modulation of light intensity was a feasible strategy for H_2 production under anoxygenic photosynthesis. The highest fuel production was observed for *C. reinhardtii* (108 umol L^{-1}) at 100 μmol m^{-2} s^{-1}.

Bioethanol can be produced from microalgae species with high carbohydrate content by sugar fermentation with yeasts [124,125]. Since carbohydrates of interest are contained in the cell coverings of the microalgae, this process requires a pre-treatment of the biomass for the hydrolysis or disruption of, for example, cell walls [126,127]. Species like *Chlorella vulgaris* [127], *Porphyridium cruentum* [124], and *Tetradesmus obliquus* [128] have been used to produce bioethanol, with yields of 11.7 g^{-1}, 2.98 mg mL^{-1}, and 11.7 g L^{-1}, respectively.

Reyimu et al. [97] studied the cultivation in batch of *Nannochloropsis oculata* and *Tetraselmis suecica* in municipal wastewater for bioethanol production. The authors showed that *T. suecica* biomass was more suitable for ethanol production, having a higher carbohydrate concentration at 7.26%. Walls et al. [95] cultured a microalgae–yeast consortium on municipal wastewater; the yeast performed aerobic fermentation, allowing for integrated WW treatment and bioethanol production with an ethanol yield of 25%.

Ferreira et al. [98] demonstrated the possibility to produce biochar, bio-oil, and biogas through the pyrolysis of *Tetradesmus obliquus* biomass produced in different WWs as such urban, dairy, and brewery industries, and cattle and poultry breeding. The bio-oil obtained showed yields in the range 30–60% (*w*/*w*) and revealed the presence of a high content of aromatic compounds. The biomass grown in brewery WW allowed the extraction of bio-oil (64%), biochar (30%), and biogas (6%).

Biofuel production is often the chosen application for microalgal biomass, as it uses well established technologies and is not impaired by the possible/probable contaminants present. Considering the pressure to find alternative processes for energy production to replace the use of fossil fuels, it is undoubtedly useful. However, biofuels are low commodities and the revenue obtained might not be enough to offset the costs associated with wastewater treatment. The following sections will, therefore, focus on other applications that might be able to produce higher revenues.

4.2. Biofertilizers and Bioestimulants

Chemical fertilizers are substances with high concentrations of available nutrients and have been used exponentially since the 1950s [129]. The overapplication of fertilizers has led to eutrophication, softening of plant tissues, and reduced root colonization due to oversupply of nitrogen, significantly increasing global greenhouse gas emissions [130]. Because of this, biofertilizers have been increasingly recommended because they can improve the biological and physical state of the plants and soil. Biofertilizers include micro- and macro-organisms that can improve the growth of plants through the colonization of the soil, rhizosphere, or the interior of the plant [131–134]. Biostimulants are products derived from organic material, which contain bioactive compounds and have the capacity to regulate and improve the physiological processes of crops or soil [135–137]. An increasing interest in biostimulants has been seen over the years, mainly because they are a cost-effective alternative to the use of chemical products and they are considered a way to increase crop productivity [135].

The role of cyanobacterial and microalgal biomass as a biofertilizer has been well established in the agricultural sector. Microalgae biomass contains higher amounts of nitrogen, whereas cyanobacteria biomass has a higher ability for nitrogen fixation [138]. Other benefits include: (i) growth promoters through the release of growth hormones, amino acids, and polysaccharides for plants [10]; (ii) biocontrol of agricultural pests and promotion of antagonism and biological control of phytopathogenic organisms [133]; (iii) increased soil fertility [134]; (iv) reduction of soil erosion through the regulation of water flow; (v) reduction of energy consumption and contamination of soil and water bodies; (vi) increased crop productivity per area; (vii) nitrogen-fixation ability by cyanobacteria, which can be used by higher plants; and (viii) renewable solutions for chemical fertilizers [132]. Several types of biofertilizers have been formulated, either as carrier-based, liquid formulations, or pellets. However, there are still some constraints on the commercial use of microalgal biofertilizers such as abiotic and biotic stresses, climate factors, and finding a suitable carrier for the cultures [131].

Several studies have revealed that biostimulants from microalgae are able to accelerate seed germination [139,140]; *Tetradesmus dimorphus*, for example, improved nutrient uptake in tomato plants [134], while *Chlorella vulgaris* and *Tetradesmus quadricauda* upregulated the expression of genes related to nutrient acquisition in sugar beet [136]. *Tetradesmus obliquus* grown in brewery wastewater increased the germination index of watercress seeds by 40% when using the biomass at 0.1 g L^{-1}, without any pre-treatment [141]. However, according to Chiaiese et al. [129], microalgal biostimulants are usually intracellular compounds and, therefore, an extraction should be performed to increase their bioavailability to plants. Microalgal biomass and/or extracts are also rich in amino acids, which are known to have a positive effect on the growth and yield of the plant and in lessening the effects of abiotic stress [140]. Amino acids like tryptophan and arginine are known metabolic precursors of phytohormones such as auxins and salicylic acid and polyamines, respectively [121]. Even though biostimulants show a diversity of potential benefits, there is still limited evidence about the interaction between microalgae and crops, and application parameters need to be optimized [121]. However, different products are already commercially available (e.g., AgriAlgae®) and microalgal extracts are already being included in new formulations of biostimulants.

The high costs of biomass production related with process scale up, and biomass harvest and processing are important challenges for the valorization of microalgal biomass as biofertilizers. However, the use of wastewater as a nutrient source for biomass production can off-set an important part of these production costs. Hence, if the safety of this biomass can be assured, its use as biofertilizer could be an alternative for a cost-effective wastewater treatment process. This would also contribute to closing the nutrient cycle, as the nitrogen would be fixed in microalgal biomass and then slowly released to be used by plants. When combining microalgae production of biofertilizers or biostimulants with wastewater treatment, it is essential to assess the chemical safety of the microalgal biomass, due to the ability of the cells to absorb heavy metals and other pollutants, such as pesticides, pharmaceuticals, plasticizers, personal care products or even hormonal residues.

4.3. High-Value Chemicals

Typically, microalgae are grown in wastewater with two main objectives: to treat water by the removal of nutrients and, simultaneously, to produce proteins, lipids, and carbohydrates that could be used in different applications, in a more sustainable way [142]. However, microalgae are also a promising source of other added-value compounds with high potential for biotechnological applications, namely pigments, fatty acids, and antioxidants [143]. Pigments, which include chlorophylls, carotenoids, and phycobilins, play a very important role in the cell, as they are used in the process of photosynthesis and possess photoprotection properties against saturating light [144–147]. They are mainly used as food colorants and vitamins for the cosmetics and pharmaceutical industries [148,149]. Chlorophyllins, which are derivatives of chlorophyll in which the magnesium is replaced by sodium or copper, have been used as valuable natural colorants, as they are safer than their synthetic counterparts, and as dietary supplements, because of their anti-inflammatory, antibacterial, and anticarcinogenic activities [150].

Pigments are used in fluorescent-based detection systems due to their absorbance spectrum properties; these include fluorescent markers and dyes for molecular biology, for labelling antibodies, and for flow cytometry applications [149]. Carotenoids have an important role in human nutrition, as they are precursors for vitamin A and powerful and efficient antioxidants [149]. Because of their photoprotective action, they are used as food and feed additives [148]. They exhibit antioxidant, antiaging, and anti-inflammatory activities, and they can display immunostimulating properties and contribute to the prevention of diabetes, cardiac, and cancer diseases [148–150]. *Haematococcus pluvialis* astaxanthin [135], *Dunaliella salina* β-carotene [149], *Haloferax alexandrinus* canthaxanthin [145], *Muriellopsis* sp. lutein [146], and *Isochrysis galbana* fucoxanthin [147] are part of the list of carotenoids in high demand that are produced by microalgae. Ultimately, this results in an increase in demand for natural carotenoids, driving major efforts to improve their production from biological sources. Ajijah et al. [148] studied tofu wastewater treatment by *Chlorella vulgaris* and *Arthrospira platensis* and observed that the microalgae growing in a medium of 10% wastewater yielded 72.2 mg/L of carotenoids after a 10-day incubation.

Polyunsaturated fatty acids (PUFA) are fatty acids with more than one double bond. It has been proved that they are essential for human health, as they have several key properties, such as antioxidant and anti-inflammatory activities, and are able to prevent several diseases, such as coronary heart problems, depression, dementia, Alzheimer's, and allergies. Vertebrates can synthesize the majority of the necessary PUFAs, except for the precursors of the biosynthesis of n-3 and n-6 PUFA, namely α-linoleic acid (ALA) and linoleic acid (LA) [150].

Because of this, n-3 and n-6 PUFA must be supplemented through diet. Among these PUFAs, there are two that are highly valuable: docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5), which is the precursor of DHA. DHA has been defined as a primary structural component for the central nervous system and EPA has been found to have a beneficial impact on several types of disfunctions [149]. The search for sustainable sources of these high-value compounds has increased and microalgae are strong candidates since they produce and accumulate PUFA even when cultivated in wastewater [142]. Jung and Lovitt [151] investigated the culture of *Aurantiochytrium limacinum* SR21 for removal of aquaculture wastewater nutrients and PUFA production. The production of long chain fatty acids was enhanced when algae were grown with wastewater supplemented with yeast extract and glycerol.

Phenols and flavonoids can also be obtained from the produced biomass after treating the effluent with subcritical water extraction (SWE) at temperatures above 120 °C, with high efficiencies from brewery WW reported by Ferreira et al. [14]: (a) phenols (0.249–1.016 mg_{GAE} mL^{-1}) and flavonoids (0.05–0.167 mg_{CE} mL^{-1}). The drastic conditions of extraction (high temperatures, up to 220 °C, 30 bar pressure, and 20 min of extraction time) can cause the elimination/lysis of several bacteria, including pathogens present in the biomass [14], which represents an advantage of SWE when applied on wastewater-grown microalgal biomass.

Extraction of high value compounds may be very important in achieving cost-effectiveness in microalgae WW treatment, especially when compared with production of biofuels, which are a low commodity. However, the sustainability of high value product extraction (e.g., use of non-toxic organic solvents or reusable solvents) also needs to be addressed.

5. Main Challenges in Wastewater Treatment by Microalgae

In microalgal wastewater treatment, the interaction between wastewater composition and microalgae is the focal point, as previously mentioned. It is unlikely that wastewater composition will be compatible with the optimal balance of nutrients needed for cell growth, even though compounds such as nitrogen, phosphorus, and carbon are harnessed for cell growth and proliferation. Depending on the emitting source, there will be variation in the composition of wastewater. which also includes different pollutants and compounds that may be toxic to microalgal cultivation depending on the amounts in which they are present. Possible ways to overcome this are, for example, the use of microorganisms isolated from wastewater and the modification of wastewater composition (e.g., pre-treatment) to satisfy some microalgal growth conditions.

The main characteristics of microalgal strains for wastewater treatment should be adaptability to environmental conditions and harmful compounds from the WW, high nutrient removal rate, and high productivity. These characteristics are what basically define a robust system with a high potential for scale-up. Since the focus of the system is the WW treatment itself, the biomass composition is not a major factor and it will vary according to wastewater composition, as well as the fluctuation of abiotic and biotic factors in systems implemented outdoors. However, the variation of the biomass composition should be followed, as it will determine its application and processing.

The composition of WW varies according to its origin as well as the time of year. Some WWs contain high concentrations of organic carbon, nitrogen, phosphorus, and toxic compounds, and cannot be directly used in the cultivation of microalgae. For this reason, some steps of pre-treatment of the wastewater should be carried out to reduce nutritional and solid loads, for example, through physical (filtration), chemical (coagulation, flocculation), and biological (anaerobic digestion or aerobic stabilization of organic matter) treatments. The C:N and N:P ratios are important points in the assimilation of nutrients, so the selection and optimization of the pre-treatment methods, such as electrocoagulation, ammonia stripping, photo-Fenton, and constructed wetlands, must be carried out according to the WW characteristics. In addition to other environmental factors, pH control is important in the balance of these ratios since it determines the dilution and availability of nutrients in the culture medium for microalgae [10].

Several strains have been studied for the treatment of effluents. The best results have been found with microalgae that have been previously conditioned to the compounds present in the WW, but mainly from strains isolated from the WW itself. Isolated microalgae are naturally adapted to weather conditions as well as WW composition and have high resistance to the harmful pollutants present in such an environment. Other factors such as pH and temperature, light intensity, light and dark cycle, carbon:nitrogen ratio, nitrogen:phosphorus ratio, CO_2 supplementation, and cultivation modes can significantly affect the wastewater treatment capacity of microalgae and, consequently, their productivity [152]. Another alternative is the use of consortia of microorganisms naturally present in WW or blooms. Among these microorganisms, in addition to microalgae of different species, bacteria, protozoa, and other organisms can be found. Bacteria can assist in WW treatment as they can serve as microalgae protectors for toxic compounds because they increase the removal of contaminants. Microalgae use CO_2 through photosynthesis to generate O_2, which can be used by heterotrophic bacteria to assimilate and degrade carbon, nitrogen, and phosphorus. In addition, the CO_2, nitrogen, and phosphorus released by bacterial aerobic metabolism can be used by microalgae for photosynthesis.

The development of specific bioreactors for microalgae wastewater treatment is another important issue that will determine the exposure of cells to light, as well as the circulation of nutrients. Light availability is one of the essential factors for the efficiency of WW treatment. Although under outdoor conditions, light cannot be fully controlled, the choice of a material with transparency and little adhesion properties to prevent biofilm deposition is essential so that there is no reduction in light penetration. The energy consumed for aeration, culture mixing, and WW feeding can be reduced by optimizing the configuration and mode of operation of the bioreactors [142]. The main challenge for industrialization and commercialization of an integrated microalgae system for WW treatment is the cost of system installation and operation.

Despite the variety of studies focused on different types of effluents from varied sources, most of them are done at the laboratory scale, where the behavior of the system can be totally different from the pilot and industrial scales. The evaluation of the economic viability of WW treatment systems under real operating conditions is still necessary, always observing the particularities of a given system, which will vary according to the source of the WW and the characteristics of the microorganisms to be used in the treatment. The cost of the PBRs themselves, including the materials for the construction of the reactor, which must be cheap and durable with low attenuation of light, must be taken into consideration. Another way of reducing costs is system automation, which can increase the efficiency of the treatment and productivity by controlling the cultivation conditions and decreasing labor costs [10,17,152].

WW treatment with microalgae can contribute to a circular economy, a system where all steps of a process are connected in order to re-use and add value to waste and raw material (Figure 4). In urban systems for example, the produced biomass is rich in carbohydrates and proteins that can be applied in energy production, which in turn can be used as an energy resource for the WWTP. The same concept can be applied to in industrial WW treatment. In agroindustry, besides the application of the biomass for animal feed, water can be reused for plant irrigation. In aquaculture, the treated water can return to fish rearing while the biomass can be used for fish feed production. These alternatives can turn WW treatment by microalgae into an even more sustainable zero waste process.

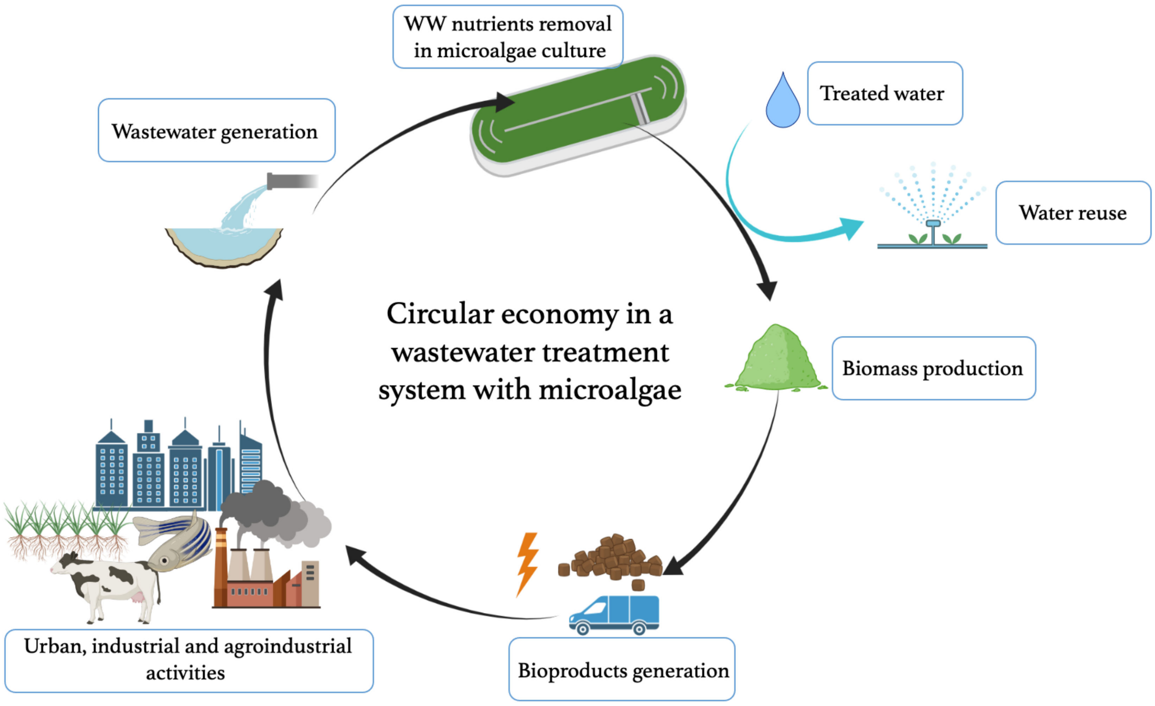

Figure 4. Circular economy in a wastewater treatment system with microalgae. This figure was made with BioRender.

6. Final Considerations

The use of microalgae to treat WW can be economically feasible, due to the positive net energy balances that have been reported; however, more studies at the and pilot scales to assess process costs are still needed. Treatment efficiency can be improved by using specific microalgal strains and/or natural consortia. Biofuel production is one of the main sources of income for biomass produced in wastewater. Depending on the composition of the different effluents that microalgae can treat, a variety of biofuels can be obtained. However, it is equally important to determine the chemical composition and presence of toxic compounds in the microalgal biomass, especially if used as feedstock for biostimulant, biofertilizer, or animal feed production, all applications that are extremely important to feeding the starved and growing population. These strategies promote not only wastewater treatment, but also upgrade biomass value, leading to more sustainable WW treatment processes in the near future.

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References

1. Urban Waste Water Treatment in Europe. Available online: https://www.eea.europa.eu/data-and-maps/indicators/urban-waste-water-treatment/urban-waste-water-treatment-assessment-5 (accessed on 19 November 2021).
2. United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000215644 (accessed on 19 November 2021).
3. Shahid, A.; Malik, S.; Zhu, H.; Xu, J.; Nawaz, M.Z.; Nawaz, S.; Asraful Alam, M.; Mehmood, M.A. Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Sci. Total Environ.* **2020**, *704*, 135303. [CrossRef]
4. Kurade, M.B.; Kim, J.R.; Govindwar, S.P.; Jeon, B.H. Insights into microalgae mediated biodegradation of diazinon by Chlorella vulgaris: Microalgal tolerance to xenobiotic pollutants and metabolism. *Algal Res.* **2016**, *20*, 126–134. [CrossRef]
5. Xiong, J.Q.; Kurade, M.B.; Jeon, B.H. Ecotoxicological effects of enrofloxacin and its removal by monoculture of microalgal species and their consortium. *Environ. Pollut.* **2017**, *226*, 486–493. [CrossRef] [PubMed]
6. Markou, G.; Wang, L.; Ye, J.; Unc, A. Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns. *Biotechnol. Adv.* **2018**, *36*, 1238–1254. [CrossRef] [PubMed]
7. Markou, G.; Vandamme, D.; Muylaert, K. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Res.* **2014**, *65*, 186–202. [CrossRef] [PubMed]
8. Li, K.; Liu, Q.; Fang, F.; Luo, R.; Lu, Q.; Zhou, W.; Huo, S.; Cheng, P.; Liu, J.; Addy, M.; et al. Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresour. Technol.* **2019**, *291*, 121934. [CrossRef]
9. Posadas, E.; Bochon, S.; Coca, M.; García-González, M.C.; García-Encina, P.A.; Muñoz, R. Microalgae-based agro-industrial wastewater treatment: A preliminary screening of biodegradability. *J. Appl. Phycol.* **2014**, *26*, 2335–2345. [CrossRef]
10. Acién Fernández, F.G.; Gómez-Serrano, C.; Fernández-Sevilla, J.M. Recovery of Nutrients From Wastewaters Using Microalgae. *Front. Sustain. Food Syst.* **2018**, *2*, 59. [CrossRef]
11. Bussa, M.; Eisen, A.; Zollfrank, C.; Röder, H. Life cycle assessment of microalgae products: State of the art and their potential for the production of polylactid acid. *J. Clean. Prod.* **2019**, *213*, 1299–1312. [CrossRef]
12. Mennaa, F.Z.; Arbib, Z.; Perales, J.A. Urban wastewater treatment by seven species of microalgae and analgal bloom: Biomass production, N and P removal kinetics andharvestability. *Water Res.* **2015**, *83*, 42–51. [CrossRef] [PubMed]
13. Schulze, P.S.C.; Carvalho, C.F.M.; Pereira, H.; Gangadhar, K.N.; Schüler, L.M.; Santos, T.F.; Varela, J.C.S.; Barreira, L. Urban wastewater treatment by Tetraselmis sp. CTP4 (Chlorophyta). *Bioresour. Technol.* **2017**, *223*, 175–183. [CrossRef] [PubMed]
14. Ferreira, A.; Ribeiro, B.; Ferreira, A.F.; Tavares, M.L.A.; Vladic, J.; Vidović, S.; Cvetkovic, D.; Melkonyan, L.; Avetisova, G.; Goginyan, V.; et al. Scenedesmus obliquus microalga-based biorefinery—From brewery effluent to bioactive compounds, biofuels and biofertilizers—Aiming at a circular bioeconomy. *Biofuels Bioprod. Biorefin.* **2019**, *13*, 1169–1186. [CrossRef]
15. Posadas, E.; Alcántara, C.; García-Encina, P.A.; Gouveia, L.; Guieysse, B.; Norvill, Z.; Acién, F.G.; Markou, G.; Congestri, R.; Koreivienė, J.; et al. Microalgae cultivation in wastewater. In *Microalgae-Based Biofuels and Bioproducts*; Gonzalez, C., Munoz, R., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; ISBN 9780081010235. [CrossRef]
16. Arun, J.; Gopinath, K.P.; SundarRajan, P.S.; Felix, V.; JoselynMonica, M.; Malolan, R. A conceptual review on microalgae biorefinery through thermochemical and biological pathways: Bio-circular approach on carbon capture and wastewater treatment. *Bioresour. Technol. Rep.* **2020**, *11*, 100477. [CrossRef]
17. Gouveia, L.; Graça, S.; Sousa, C.; Ambrosano, L.; Ribeiro, B.; Botrel, E.P.; Neto, P.C.; Ferreira, A.F.; Silva, C.M. Microalgae biomass production using wastewater: Treatment and costs. Scale-up considerations. *Algal Res.* **2016**, *16*, 167–176. [CrossRef]
18. Arbib, Z.; Ruiz, J.; Álvarez-Díaz, P.; Garrido-Pérez, C.; Perales, J.A. Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO_2 bio-fixation and low cost biofuels production. *Water Res.* **2014**, *49*, 465–474. [CrossRef]
19. Deblonde, T.; Cossu-Leguille, C.; Hartemann, P. Emerging pollutants in wastewater: A review of the literature. *Int. J. Hyg. Environ. Health* **2011**, *214*, 442–448. [CrossRef] [PubMed]
20. Santos, L.H.M.L.M.; Gros, M.; Rodriguez-Mozaz, S.; Delerue-Matos, C.; Pena, A.; Barceló, D.; Montenegro, M.C.B.S.M. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. *Sci. Total Environ.* **2013**, *461–462*, 302–316. [CrossRef]
21. Ferreira, A.; Reis, A.; Vidovic, S.; Vladic, J.; Gkelis, S.; Melkonyan, L.; Avetisova, G.; Congestri, R.; Acién, G.; Muñoz, R.; et al. Combining Microalgae-Based Wastewater Treatment with Biofuel and Bio-Based Production in the Frame of a Biorefinery. In *Grand Challenges in Biology and Biotechnology*; Springer: Cham, Switzerland, 2019; pp. 319–369.
22. García, D.; Posadas, E.; Blanco, S.; Acién, G.; García-Encina, P.; Bolado, S.; Muñoz, R. Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors. *Bioresour. Technol.* **2018**, *248*, 120–126. [CrossRef]

23. Ferreira, A.; Melkonyan, L.; Carapinha, S.; Ribeiro, B.; Figueiredo, D.; Avetisova, G.; Gouveia, L. Biostimulant and biopesticide potential of microalgae growing in piggery wastewater. *Environ. Adv.* **2021**, *4*, 100062. [CrossRef]
24. Blanchard, M.; Teil, M.J.; Ollivon, D.; Legenti, L.; Chevreuil, M. Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewaters and sewage sludges from the Paris area (France). *Environ. Res.* **2004**, *95*, 184–197. [CrossRef]
25. Verlicchi, P.; Galletti, A.; Petrovic, M.; BarcelÓ, D. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. *J. Hydrol.* **2010**, *389*, 416–428. [CrossRef]
26. Xie, P.; Ho, S.H.; Peng, J.; Xu, X.J.; Chen, C.; Zhang, Z.F.; Lee, D.J.; Ren, N.Q. Dual purpose microalgae-based biorefinery for treating pharmaceuticals and personal care products (PPCPs) residues and biodiesel production. *Sci. Total Environ.* **2019**, *688*, 253–261. [CrossRef] [PubMed]
27. Posadas, E.; Morales, M. del M.; Gomez, C.; Acién, F.G.; Muñoz, R. Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. *Chem. Eng. J.* **2015**, *265*, 239–248. [CrossRef]
28. Mennaa, F.Z.; Arbib, Z.; Perales, J.A. Urban wastewater photobiotreatment with microalgae in a continuously operated photobioreactor: Growth, nutrient removal kinetics and biomass coagulation–flocculation. *Environ. Technol.* **2019**, *40*, 342–355. [CrossRef]
29. Foladori, P.; Petrini, S.; Andreottola, G. Evolution of real municipal wastewater treatment in photobioreactors and microalgae-bacteria consortia using real-time parameters. *Chem. Eng. J.* **2018**, *345*, 507–516. [CrossRef]
30. Arias, D.M.; Solé-Bundó, M.; Garfí, M.; Ferrer, I.; García, J.; Uggetti, E. Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. *Bioresour. Technol.* **2018**, *247*, 513–519. [CrossRef]
31. Gutiérrez, R.; Ferrer, I.; González-Molina, A.; Salvadó, H.; García, J.; Uggetti, E. Microalgae recycling improves biomass recovery from wastewater treatment high rate algal ponds. *Water Res.* **2016**, *106*, 539–549. [CrossRef] [PubMed]
32. Caporgno, M.P.; Taleb, A.; Olkiewicz, M.; Font, J.; Pruvost, J.; Legrand, J.; Bengoa, C. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. *Algal Res.* **2015**, *10*, 232–239. [CrossRef]
33. De Alva, M.S.; Luna-Pabello, V.M.; Cadena, E.; Ortíz, E. Green microalga Scenedesmus acutus grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. *Bioresour. Technol.* **2013**, *146*, 744–748. [CrossRef]
34. Hom-Diaz, A.; Jaén-Gil, A.; Bello-Laserna, I.; Rodríguez-Mozaz, S.; Vicent, T.; Barceló, D.; Blánquez, P. Performance of a microalgal photobioreactor treating toilet wastewater: Pharmaceutically active compound removal and biomass harvesting. *Sci. Total Environ.* **2017**, *592*, 1–11. [CrossRef]
35. Muñoz, I.; José Gómez, M.; Molina-Díaz, A.; Huijbregts, M.A.J.; Fernández-Alba, A.R.; García-Calvo, E. Ranking potential impacts of priority and emerging pollutants in urban wastewater through life cycle impact assessment. *Chemosphere* **2008**, *74*, 37–44. [CrossRef] [PubMed]
36. Maranho, L.A.; Garrido-Pérez, M.C.; Delvalls, T.A.; Martín-Díaz, M.L. Suitability of standardized acute toxicity tests for marine sediment assessment: Pharmaceutical contamination. *Water. Air. Soil Pollut.* **2015**, *226*. [CrossRef]
37. Pereira, A.M.P.T.; Silva, L.J.G.; Meisel, L.M.; Lino, C.M.; Pena, A. Environmental impact of pharmaceuticals from Portuguese wastewaters: Geographical and seasonal occurrence, removal and risk assessment. *Environ. Res.* **2015**, *136*, 108–119. [CrossRef]
38. Gonzalez-Rey, M.; Bebianno, M.J. Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel Mytilus galloprovincialis? *Environ. Toxicol. Pharmacol.* **2012**, *33*, 361–371. [CrossRef]
39. Guo, W.Q.; Zheng, H.S.; Li, S.; Du, J.S.; Feng, X.C.; Yin, R.L.; Wu, Q.L.; Ren, N.Q.; Chang, J.S. Removal of cephalosporin antibiotics 7-ACA from wastewater during the cultivation of lipid-accumulating microalgae. *Bioresour. Technol.* **2016**, *221*, 284–290. [CrossRef]
40. Danovaro, R.; Bongiorni, L.; Corinaldesi, C.; Giovannelli, D.; Damiani, E.; Astolfi, P.; Greci, L.; Pusceddu, A. Sunscreens Cause Coral Bleaching by Promoting Viral Infections. *Environ. Health Perspect.* **2008**, *116*, 441–447. [CrossRef]
41. Li, S.; He, B.; Wang, J.; Liu, J.; Hu, X. Risks of caffeine residues in the environment: Necessity for a targeted ecopharmacovigilance program. *Chemosphere* **2020**, *243*, 125343. [CrossRef] [PubMed]
42. Wang, Y.; Liu, J.; Kang, D.; Wu, C.; Wu, Y. Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: A review. *Rev. Environ. Sci. Biotechnol.* **2017**, *16*, 717–735. [CrossRef]
43. DeLorenzo, M.E.; Fleming, J. Individual and Mixture Effects of Selected Pharmaceuticals and Personal Care Products on the Marine Phytoplankton Species Dunaliella tertiolecta. *Arch. Environ. Contam. Toxicol.* **2008**, *54*, 203–210. [CrossRef]
44. Cuerda-Correa, E.M.; Alexandre-Franco, M.F.; Fernández-González, C. Advanced Oxidation Processes for the Removal of Antibiotics from Water. An Overview. *Water* **2020**, *12*, 102. [CrossRef]
45. Xiong, J.Q.; Kurade, M.B.; Jeon, B.H. Can Microalgae Remove Pharmaceutical Contaminants from Water? *Trends Biotechnol.* **2018**, *36*, 30–44. [CrossRef]
46. Gentili, F.G.; Fick, J. Algal cultivation in urban wastewater: An efficient way to reduce pharmaceutical pollutants. *J. Appl. Phycol.* **2017**, *29*, 255–262. [CrossRef]
47. López-Serna, R.; García, D.; Bolado, S.; Jiménez, J.J.; Lai, F.Y.; Golovko, O.; Gago-Ferrero, P.; Ahrens, L.; Wiberg, K.; Muñoz, R. Photobioreactors based on microalgae-bacteria and purple phototrophic bacteria consortia: A promising technology to reduce the load of veterinary drugs from piggery wastewater. *Sci. Total Environ.* **2019**, *692*, 259–266. [CrossRef] [PubMed]
48. De Wilt, A.; Butkovskyi, A.; Tuantet, K.; Leal, L.H.; Fernandes, T.V.; Langenhoff, A.; Zeeman, G. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *J. Hazard. Mater.* **2016**, *304*, 84–92. [CrossRef]

49. Escapa, C.; Coimbra, R.N.; Paniagua, S.; García, A.I.; Otero, M. Nutrients and pharmaceuticals removal from wastewater by culture and harvesting of Chlorella sorokiniana. *Bioresour. Technol.* **2015**, *185*, 276–284. [CrossRef]
50. Escapa, C.; Coimbra, R.N.; Paniagua, S.; García, A.I.; Otero, M. Comparative assessment of diclofenac removal from water by different microalgae strains. *Algal Res.* **2016**, *18*, 127–134. [CrossRef]
51. Matamoros, V.; Gutiérrez, R.; Ferrer, I.; García, J.; Bayona, J.M. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *J. Hazard. Mater.* **2015**, *288*, 34–42. [CrossRef]
52. Xiong, J.-Q.; Kurade, M.B.; Abou-Shanab, R.A.I.; Ji, M.-K.; Choi, J.; Kim, J.O.; Jeon, B.-H. Biodegradation of carbamazepine using freshwater microalgae Chlamydomonas mexicana and Scenedesmus obliquus and the determination of its metabolic fate. *Bioresour. Technol.* **2016**, *205*, 183–190. [CrossRef] [PubMed]
53. Colunga, A.; Rangel-Mendez, J.R.; Celis, L.B.; Cervantes, F.J. Graphene oxide as electron shuttle for increased redox conversion of contaminants under methanogenic and sulfate-reducing conditions. *Bioresour. Technol.* **2015**, *175*, 309–314. [CrossRef]
54. Zhang, J.; Fu, D.; Wu, J. Photodegradation of Norfloxacin in aqueous solution containing algae. *J. Environ. Sci.* **2012**, *24*, 743–749. [CrossRef]
55. Della Greca, M.; Pinto, G.; Pistillo, P.; Pollio, A.; Previtera, L.; Temussi, F. Biotransformation of ethinylestradiol by microalgae. *Chemosphere* **2008**, *70*, 2047–2053. [CrossRef]
56. Xiong, J.-Q.; Govindwar, S.; Kurade, M.B.; Paeng, K.-J.; Roh, H.-S.; Khan, M.A.; Jeon, B.-H. Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, Scenedesmus obliquus. *Chemosphere* **2019**, *218*, 551–558. [CrossRef]
57. Xiong, Q.; Liu, Y.-S.; Hu, L.-X.; Shi, Z.-Q.; Cai, W.-W.; He, L.-Y.; Ying, G.-G. Co-metabolism of sulfamethoxazole by a freshwater microalga Chlorella pyrenoidosa. *Water Res.* **2020**, *175*, 115656. [CrossRef]
58. Bai, X.; Acharya, K. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga Nannochloris sp. *J. Hazard. Mater.* **2016**, *315*, 70–75. [CrossRef] [PubMed]
59. Peng, F.Q.; Ying, G.G.; Yang, B.; Liu, S.; Lai, H.J.; Liu, Y.S.; Chen, Z.F.; Zhou, G.J. Biotransformation of progesterone and norgestrel by two freshwater microalgae (Scenedesmus obliquus and Chlorella pyrenoidosa): Transformation kinetics and products identification. *Chemosphere* **2014**, *95*, 581–588. [CrossRef]
60. Hena, S.; Gutierrez, L.; Croué, J.-P. Removal of metronidazole from aqueous media by *C. vulgaris*. *J. Hazard. Mater.* **2020**, *384*, 121400. [CrossRef]
61. Ferreira, A.; Marques, P.; Ribeiro, B.; Assemany, P.; de Mendonça, H.V.; Barata, A.; Oliveira, A.C.; Reis, A.; Pinheiro, H.M.; Gouveia, L. Combining biotechnology with circular bioeconomy: From poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. *Environ. Res.* **2018**, *164*, 32–38. [CrossRef] [PubMed]
62. Muñoz, R.; Guieysse, B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Res.* **2006**, *40*, 2799–2815. [CrossRef]
63. Cai, T.; Park, S.Y.; Li, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renew. Sustain. Energy Rev.* **2013**, *19*, 360–369. [CrossRef]
64. Hernández, D.; Riaño, B.; Coca, M.; García-González, M.C. Treatment of agro-industrial wastewater using microalgae–bacteria consortium combined with anaerobic digestion of the produced biomass. *Bioresour. Technol.* **2013**, *135*, 598–603. [CrossRef]
65. Wang, H.; Xiong, H.; Hui, Z.; Zeng, X. Mixotrophic cultivation of Chlorella pyrenoidosa with diluted primary piggery wastewater to produce lipids. *Bioresour. Technol.* **2012**, *104*, 215–220. [CrossRef]
66. Qi, F.; Xu, Y.; Yu, Y.; Liang, X.; Zhang, L.; Zhao, H.; Wang, H. Enhancing growth of Chlamydomonas reinhardtii and nutrient removal in diluted primary piggery wastewater by elevated CO_2 supply. *Water Sci. Technol.* **2017**, *75*, 2281–2290. [CrossRef]
67. Cañizares, R.O.; Domínguez, A.R. Growth of Spirulina maxima on swine waste. *Bioresour. Technol.* **1993**, *45*, 73–75. [CrossRef]
68. Dos Santos, A.M.; Roso, G.R.; de Menezes, C.R.; Queiroz, M.I.; Zepka, L.Q.; Jacob-Lopes, E. The bioeconomy of microalgal heterotrophic bioreactors applied to agroindustrial wastewater treatment. *Desalin. Water Treat.* **2017**, *64*, 12–20. [CrossRef]
69. Tsolcha, O.N.; Tekerlekopoulou, A.G.; Akratos, C.S.; Aggelis, G.; Genitsaris, S.; Moustaka-Gouni, M.; Vayenas, D.V. Agroindustrial wastewater treatment with simultaneous biodiesel production in attached growth systems using a mixed microbial culture. *Water* **2018**, *10*, 1693. [CrossRef]
70. Cheah, W.Y.; Show, P.L.; Juan, J.C.; Chang, J.S.; Ling, T.C. Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal. *Energy Convers. Manag.* **2018**, *174*, 430–438. [CrossRef]
71. Phang, S.M.; Miah, M.S.; Yeoh, B.G.; Hashim, M.A. Spirulina cultivation in digested sago starch factory wastewater. *J. Appl. Phycol.* **2000**, *12*, 395–400. [CrossRef]
72. Kamarudin, K.F.; Yaakob, Z.; Rajkumar, R.; Tasirin, S.M. Bioremediation of palm oil mill effluents (POME) using Scenedesmus dimorphus and chlorella vulgaris. *Adv. Sci. Lett.* **2013**, *19*, 2914–2918. [CrossRef]
73. Ding, G.T.; Yaakob, Z.; Takriff, M.S.; Salihon, J.; Abd Rahaman, M.S. Biomass production and nutrients removal by a newly-isolated microalgal strain Chlamydomonas sp in palm oil mill effluent (POME). *Int. J. Hydrogen Energy* **2016**, *41*, 4888–4895. [CrossRef]
74. Dias, C.; Gouveia, L.; Santos, J.A.L.; Reis, A.; Lopes da Silva, T. Using flow cytometry to monitor the stress response of yeast and microalgae populations in mixed cultures developed in brewery effluents. *J. Appl. Phycol.* **2020**, *32*, 3687–3701. [CrossRef]
75. Zhang, Y.; Habteselassie, M.Y.; Resurreccion, E.P.; Mantripragada, V.; Peng, S.; Bauer, S.; Colosi, L.M. Evaluating removal of steroid estrogens by a model alga as a possible sustainability benefit of hypothetical integrated algae cultivation and wastewater treatment systems. *ACS Sustain. Chem. Eng.* **2014**, *2*, 2544–2553. [CrossRef]

76. Massé, D.I.; Saady, N.M.C.; Gilbert, Y. Potential of biological processes to eliminate antibiotics in livestock manure: An overview. *Animals* **2014**, *4*, 146–163. [CrossRef]
77. Dosnon-Olette, R.; Trotel-Aziz, P.; Couderchet, M.; Eullaffroy, P. Fungicides and herbicide removal in Scenedesmus cell suspensions. *Chemosphere* **2010**, *79*, 117–123. [CrossRef]
78. Hom-Diaz, A.; Llorca, M.; Rodríguez-Mozaz, S.; Vicent, T.; Barceló, D.; Blánquez, P. Microalgae cultivation on wastewater digestate: β-estradiol and 17α-ethynylestradiol degradation and transformation products identification. *J. Environ. Manag.* **2015**, *155*, 106–113. [CrossRef]
79. Mehta, S.K.; Gaur, J.P. Characterization and optimization of Ni and Cu sorption from aqueous solution by *Chlorella vulgaris*. *Ecol. Eng.* **2001**, *18*, 1–13. [CrossRef]
80. Industrial Waste in Europe. Available online: https://www.eea.europa.eu/themes/industry (accessed on 19 October 2021).
81. Del Rosario Martínez-Macias, M.; Correa-Murrieta, M.A.; Villegas-Peralta, Y.; Dévora-Isiordia, G.E.; Álvarez-Sánchez, J.; Saldivar-Cabrales, J.; Sánchez-Duarte, R.G. Uptake of copper from acid mine drainage by the microalgae Nannochloropsis oculata. *Environ. Sci. Pollut. Res.* **2019**, *26*, 6311–6318. [CrossRef]
82. Romera, E.; González, F.; Ballester, A.; Blázquez, M.L.; Muñoz, J.A. Comparative study of biosorption of heavy metals using different types of algae. *Bioresour. Technol.* **2007**, *98*, 3344–3353. [CrossRef] [PubMed]
83. Khoei, A.J.; Ghaleh Joogh, N.J.; Darvishi, P.; Rezaei, K. Application of physical and biological methods to remove heavy metal, arsenic and pesticides, malathion and diazinon from water. *Turkish J. Fish. Aquat. Sci.* **2019**, *19*, 21–28. [CrossRef]
84. Shen, L.; Saky, S.A.; Yang, Z.; Ho, S.-H.; Chen, C.; Qin, L.; Zhang, G.; Wang, Y.; Lu, Y. The critical utilization of active heterotrophic microalgae for bioremoval of Cr(VI) in organics co-contaminated wastewater. *Chemosphere* **2019**, *228*, 536–544. [CrossRef]
85. Saavedra, R.; Muñoz, R.; Taboada, M.E.; Vega, M.; Bolado, S. Comparative uptake study of arsenic, boron, copper, manganese and zinc from water by different green microalgae. *Bioresour. Technol.* **2018**, *263*, 49–57. [CrossRef] [PubMed]
86. Vidyalaxmi; Kaushik, G.; Raza, K. Potential of novel Dunaliella salina from sambhar salt lake, India, for bioremediation of hexavalent chromium from aqueous effluents: An optimized green approach. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 430–438. [CrossRef]
87. Cameron, H.; Mata, M.T.; Riquelme, C. The effect of heavy metals on the viability of *Tetraselmis marina* AC16-MESO and an evaluation of the potential use of this microalga in bioremediation. *PeerJ* **2018**, *6*, e5295. [CrossRef]
88. Azimi, A.; Azari, A.; Rezakazemi, M.; Ansarpour, M. Removal of Heavy Metals from Industrial Wastewaters: A Review. *ChemBioEng Rev.* **2017**, *4*, 37–59. [CrossRef]
89. Singh, N.K.; Upadhyay, A.K.; Rai, U.N. Algal Technologies for Wastewater Treatment and Biofuels Production: An Integrated Approach for Environmental Management. In *Algal Biofuels*; Springer International Publishing: Cham, Switzerland, 2017; pp. 97–107. ISBN 9783319510101.
90. Markou, G.; Mitrogiannis, D.; Çelekli, A.; Bozkurt, H.; Georgakakis, D.; Chrysikopoulos, C.V. Biosorption of Cu^{2+} and Ni^{2+} by Arthrospira platensis with different biochemical compositions. *Chem. Eng. J.* **2015**, *259*, 806–813. [CrossRef]
91. Urrutia, C.; Yañez-Mansilla, E.; Jeison, D. Bioremoval of heavy metals from metal mine tailings water using microalgae biomass. *Algal Res.* **2019**, *43*, 101659. [CrossRef]
92. Saavedra, R.; Muñoz, R.; Taboada, M.E.; Bolado, S. Influence of organic matter and CO_2 supply on bioremediation of heavy metals by Chlorella vulgaris and Scenedesmus almeriensis in a multimetallic matrix. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109393. [CrossRef]
93. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306. [CrossRef]
94. Campenni', L.; Nobre, B.P.; Santos, C.A.; Oliveira, A.C.; Aires-Barros, M.R.; Palavra, A.M.F.; Gouveia, L. Carotenoid and lipid production by the autotrophic microalga Chlorella protothecoides under nutritional, salinity, and luminosity stress conditions. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 1383–1393. [CrossRef] [PubMed]
95. Walls, L.E.; Velasquez-Orta, S.B.; Romero-Frasca, E.; Leary, P.; Yáñez Noguez, I.; Orta Ledesma, M.T. Non-sterile heterotrophic cultivation of native wastewater yeast and microalgae for integrated municipal wastewater treatment and bioethanol production. *Biochem. Eng. J.* **2019**, *151*, 107319. [CrossRef]
96. Pereira, H.; Gangadhar, K.N.; Schulze, P.S.C.; Santos, T.; de Sousa, C.B.; Schueler, L.M.; Custódio, L.; Malcata, F.X.; Gouveia, L.; Varela, J.C.S.; et al. Isolation of a euryhaline microalgal strain, *Tetraselmis* sp. CTP4, as a robust feedstock for biodiesel production. *Sci. Rep.* **2016**, *6*, 35663. [CrossRef]
97. Reyimu, Z.; Özçimen, D. Batch cultivation of marine microalgae Nannochloropsis oculata and Tetraselmis suecica in treated municipal wastewater toward bioethanol production. *J. Clean. Prod.* **2017**, *150*, 40–46. [CrossRef]
98. Ferreira, A.F.; Ferreira, A.; Dias, A.P.S.; Gouveia, L. Pyrolysis of Scenedesmus obliquus Biomass Following the Treatment of Different Wastewaters. *Bioenergy Res.* **2020**, *13*, 896–906. [CrossRef]
99. Gouveia, L.; Oliveira, A.C. Microalgae as a raw material for biofuels production. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 269–274. [CrossRef]
100. Bartley, M.L.; Boeing, W.J.; Corcoran, A.A.; Holguin, F.O.; Schaub, T. Effects of salinity on growth and lipid accumulation of biofuel microalga Nannochloropsis salina and invading organisms. *Biomass Bioenergy* **2013**, *54*, 83–88. [CrossRef]
101. Mandal, S.; Mallick, N. Microalga Scenedesmus obliquus as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 281–291. [CrossRef] [PubMed]

102. Gouveia, L.; Marques, A.E.; Da Silva, T.L.; Reis, A. Neochloris oleabundans UTEX #1185: A suitable renewable lipid source for biofuel production. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 821–826. [CrossRef]
103. Xu, H.; Miao, X.; Wu, Q. High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. *J. Biotechnol.* **2006**, *126*, 499–507. [CrossRef] [PubMed]
104. Samorì, C.; Torri, C.; Samorì, G.; Fabbri, D.; Galletti, P.; Guerrini, F.; Pistocchi, R.; Tagliavini, E. Extraction of hydrocarbons from microalga Botryococcus braunii with switchable solvents. *Bioresour. Technol.* **2010**, *101*, 3274–3279. [CrossRef] [PubMed]
105. Gangadhar, K.N.; Pereira, H.; Diogo, H.P.; Borges dos Santos, R.M.; Prabhavathi Devi, B.L.A.; Prasad, R.B.N.; Custódio, L.; Malcata, F.X.; Varela, J.; Barreira, L. Assessment and comparison of the properties of biodiesel synthesized from three different types of wet microalgal biomass. *J. Appl. Phycol.* **2016**, *28*, 1571–1578. [CrossRef]
106. Mussgnug, J.H.; Klassen, V.; Schlüter, A.; Kruse, O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* **2010**, *150*, 51–56. [CrossRef] [PubMed]
107. Chandra, R.; Pradhan, S.; Patel, A.; Ghosh, U.K. An approach for dairy wastewater remediation using mixture of microalgae and biodiesel production for sustainable transportation. *J. Environ. Manage.* **2021**, *297*, 113210. [CrossRef]
108. Fazal, T.; Rehman, M.S.U.; Javed, F.; Akhtar, M.; Mushtaq, A.; Hafeez, A.; Alaud Din, A.; Iqbal, J.; Rashid, N.; Rehman, F. Integrating bioremediation of textile wastewater with biodiesel production using microalgae (Chlorella vulgaris). *Chemosphere* **2021**, *281*, 130758. [CrossRef] [PubMed]
109. Vargas-Estrada, L.; Longoria, A.; Okoye, P.U.; Sebastian, P.J. Energy and nutrients recovery from wastewater cultivated microalgae: Assessment of the impact of wastewater dilution on biogas yield. *Bioresour. Technol.* **2021**, *341*, 125755. [CrossRef] [PubMed]
110. Molinuevo-Salces, B.; Mahdy, A.; Ballesteros, M.; González-Fernández, C. From piggery wastewater nutrients to biogas: Microalgae biomass revalorization through anaerobic digestion. *Renew. Energy* **2016**, *96*, 1103–1110. [CrossRef]
111. Hwang, J.H.; Lee, W.H. Continuous photosynthetic biohydrogen production from acetate-rich wastewater: Influence of light intensity. *Int. J. Hydrogen Energy* **2021**, *46*, 21812–21821. [CrossRef]
112. Batista, A.P.; Ambrosano, L.; Graça, S.; Sousa, C.; Marques, P.A.S.S.; Ribeiro, B.; Botrel, E.P.; Castro Neto, P.; Gouveia, L. Combining urban wastewater treatment with biohydrogen production - An integrated microalgae-based approach. *Bioresour. Technol.* **2015**, *184*, 230–235. [CrossRef]
113. Gouveia, J.D.; Moers, A.; Griekspoor, Y.; van den Broek, L.A.M.; Springer, J.; Sijtsma, L.; Sipkema, D.; Wijffels, R.H.; Barbosa, M.J. Effect of removal of bacteria on the biomass and extracellular carbohydrate productivity of Botryococcus braunii. *J. Appl. Phycol.* **2019**, *31*, 3453–3463. [CrossRef]
114. Passos, F.; Uggetti, E.; Carrère, H.; Ferrer, I. Pretreatment of microalgae to improve biogas production: A review. *Bioresour. Technol.* **2014**, *172*, 403–412. [CrossRef] [PubMed]
115. Marques, A.E.; Barbosa, A.T.; Jotta, J.; Coelho, M.C.; Tamagnini, P.; Gouveia, L. Biohydrogen production by Anabaena sp. PCC 7120 wild-type and mutants under different conditions: Light, nickel, propane, carbon dioxide and nitrogen. *Biomass Bioenergy* **2011**, *35*, 4426–4434. [CrossRef]
116. Ferreira, A.F.; Marques, A.C.; Batista, A.P.; Marques, P.A.S.S.; Gouveia, L.; Silva, C.M. Biological hydrogen production by *Anabaena* sp.—Yield, energy and CO_2 analysis including fermentative biomass recovery. *Int. J. Hydrogen Energy* **2012**, *37*, 179–190. [CrossRef]
117. Lin, C.Y.; Nguyen, M.L.T.; Lay, C.H. Starch-containing textile wastewater treatment for biogas and microalgae biomass production. *J. Clean. Prod.* **2017**, *168*, 331–337. [CrossRef]
118. Pacheco, R.; Ferreira, A.F.; Pinto, T.; Nobre, B.P.; Loureiro, D.; Moura, P.; Gouveia, L.; Silva, C.M. The production of pigments & hydrogen through a Spirogyra sp. biorefinery. *Energy Convers. Manag.* **2015**, *89*, 789–797. [CrossRef]
119. Ortigueira, J.; Alves, L.; Gouveia, L.; Moura, P. Third generation biohydrogen production by Clostridium butyricum and adapted mixed cultures from Scenedesmus obliquus microalga biomass. *Fuel* **2015**, *153*, 128–134. [CrossRef]
120. Pinto, T.; Gouveia, L.; Ortigueira, J.; Saratale, G.D.; Moura, P. Enhancement of fermentative hydrogen production from Spirogyra sp. by increased carbohydrate accumulation and selection of the biomass pretreatment under a biorefinery model. *J. Biosci. Bioeng.* **2018**, *126*, 226–234. [CrossRef]
121. Batista, A.P.; Moura, P.; Marques, P.A.S.S.; Ortigueira, J.; Alves, L.; Gouveia, L. Scenedesmus obliquus as feedstock for biohydrogen production by Enterobacter aerogenes and Clostridium butyricum. *Fuel* **2014**, *117*, 537–543. [CrossRef]
122. Kim, M.S.; Baek, J.S.; Yun, Y.S.; Jun Sim, S.; Park, S.; Kim, S.C. Hydrogen production from Chlamydomonas reinhardtii biomass using a two-step conversion process: Anaerobic conversion and photosynthetic fermentation. *Int. J. Hydrogen Energy* **2006**, *31*, 812–816. [CrossRef]
123. Nobre, B.P.; Villalobos, F.; Barragán, B.E.; Oliveira, A.C.; Batista, A.P.; Marques, P.A.S.S.; Mendes, R.L.; Sovová, H.; Palavra, A.F.; Gouveia, L. A biorefinery from Nannochloropsis sp. microalga—Extraction of oils and pigments. Production of biohydrogen from the leftover biomass. *Bioresour. Technol.* **2013**, *135*, 128–136. [CrossRef]
124. Kim, H.M.; Oh, C.H.; Bae, H.J. Comparison of red microalgae (Porphyridium cruentum) culture conditions for bioethanol production. *Bioresour. Technol.* **2017**, *233*, 44–50. [CrossRef] [PubMed]
125. Miranda, J.R.; Passarinho, P.C.; Gouveia, L. Bioethanol production from Scenedesmus obliquus sugars: The influence of photobioreactors and culture conditions on biomass production. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 555–564. [CrossRef]
126. Harun, R.; Danquah, M.K. Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochem.* **2011**, *46*, 304–309. [CrossRef]

127. Ho, S.H.; Huang, S.W.; Chen, C.Y.; Hasunuma, T.; Kondo, A.; Chang, J.S. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. *Bioresour. Technol.* **2013**, *135*, 191–198. [CrossRef] [PubMed]
128. Miranda, J.R.; Passarinho, P.C.; Gouveia, L. Pre-treatment optimization of Scenedesmus obliquus microalga for bioethanol production. *Bioresour. Technol.* **2012**, *104*, 342–348. [CrossRef]
129. Chiaiese, P.; Corrado, G.; Colla, G.; Kyriacou, M.C.; Rouphael, Y. Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop performance. *Front. Plant Sci.* **2018**, *871*, 1782. [CrossRef] [PubMed]
130. Rose, M.T.; Phuong, T.L.; Nhan, D.K.; Cong, P.T.; Hien, N.T.; Kennedy, I.R. Up to 52% N fertilizer replaced by biofertilizer in lowland rice via farmer participatory research. *Agron. Sustain. Dev.* **2014**, *34*, 857–868. [CrossRef]
131. Win, T.T.; Barone, G.D.; Secundo, F.; Fu, P. Algal Biofertilizers and Plant Growth Stimulants for Sustainable Agriculture. *Ind. Biotechnol.* **2018**, *14*, 203–211. [CrossRef]
132. Mohan, S.V.; Hemalatha, M.; Chakraborty, D.; Chatterjee, S.; Ranadheer, P.; Kona, R. Algal biorefinery models with self-sustainable closed loop approach: Trends and prospective for blue-bioeconomy. *Bioresour. Technol.* **2020**, *295*, 122128. [CrossRef]
133. Chaudhary, V.; Prasanna, R.; Nain, L.; Dubey, S.C.; Gupta, V.; Singh, R.; Jaggi, S.; Bhatnagar, A.K. Bioefficacy of novel cyanobacteria-amended formulations in suppressing damping off disease in tomato seedlings. *World J. Microbiol. Biotechnol.* **2012**, *28*, 3301–3310. [CrossRef]
134. Garcia-Gonzalez, J.; Sommerfeld, M. Biofertilizer and biostimulant properties of the microalga Acutodesmus dimorphus. *J. Appl. Phycol.* **2016**, *28*, 1051–1061. [CrossRef]
135. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in Plant Science: A Global Perspective. *Front. Plant Sci.* **2017**, *7*, 1–32. [CrossRef] [PubMed]
136. Barone, V.; Baglieri, A.; Stevanato, P.; Broccanello, C.; Bertoldo, G.; Bertaggia, M.; Cagnin, M.; Pizzeghello, D.; Moliterni, V.M.C.; Mandolino, G.; et al. Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). *J. Appl. Phycol.* **2018**, *30*, 1061–1071. [CrossRef]
137. Arroussi, H.E.L.; Benhima, R.; Elbaouchi, A.; Sijilmassi, B.; Mernissi, N.E.L.; Aafsar, A.; Meftah-Kadmiri, I.; Bendaou, N.; Smouni, A. Dunaliella salina exopolysaccharides: A promising biostimulant for salt stress tolerance in tomato Solanum lycopersicum). *J. Appl. Phycol.* **2018**, *30*, 2929–2941. [CrossRef]
138. Abd El-Baky, H.H.; El-Baz, F.K.; El Baroty, G.S. Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *J. Sci. Food Agric.* **2010**, *90*, 299–303. [CrossRef]
139. Oancea, F.; Velea, S.; Mincea, C.; Ilie, L. Mocro-Algae based plant biostimulant and its effect on water stressed tomato plants. *Rom. J. Plant Prot.* **2013**, *4*, 104–117.
140. Gemin, L.G.; Mógor, Á.F.; Amatussi, J.D.O.; Mógor, G. Microalgae associated to humic acid as a novel biostimulant improving onion growth and yield. *Sci. Hortic.* **2019**, *256*, 108560. [CrossRef]
141. Navarro-López, E.; Ruíz-Nieto, A.; Ferreira, A.; Acién, F.G.; Gouveia, L. Biostimulant Potential of Scenedesmus obliquus Grown in Brewery Wastewater. *Molecules* **2020**, *25*, 664. [CrossRef] [PubMed]
142. Nur, M.; Buma, A.G.J. Opportunities and Challenges of Microalgal Cultivation on Wastewater, with Special Focus on Palm Oil Mill Effluent and the Production of High Value Compounds. *Waste Biomass Valoriz.* **2019**, *10*, 2079–2097. [CrossRef]
143. De Morais, M.G.; da Silva Vaz, B.; de Morais, E.G.; Costa, J.A.V. Biologically Active Metabolites Synthesized by Microalgae. *BioMed Res. Int.* **2015**, *2015*, 835761. [CrossRef] [PubMed]
144. Begum, H.; Yusoff, F.M.; Banerjee, S.; Khatoon, H.; Shariff, M. Availability and Utilization of Pigments from Microalgae. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 2209–2222. [CrossRef] [PubMed]
145. Asker, D.; Ohta, Y. Production of canthaxanthin by Haloferax alexandrinus under non-aseptic conditions and a simple, rapid method for its extraction. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 743–750. [CrossRef] [PubMed]
146. Del Campo, J.A.; Rodríguez, H.; Moreno, J.; Vargas, M.Á.; Rivas, J.; Guerrero, M.G. Lutein production by Muriellopsis sp. in an outdoor tubular photobioreactor. *J. Biotechnol.* **2001**, *85*, 289–295. [CrossRef]
147. Kim, S.M.; Kang, S.W.; Kwon, O.N.; Chung, D.; Pan, C.H. Fucoxanthin as a major carotenoid in Isochrysis aff. galbana: Characterization of extraction for commercial application. *J. Korean Soc. Appl. Biol. Chem.* **2012**, *55*, 477–483. [CrossRef]
148. Ajijah, N.; Tjandra, B.C.; Hamidah, U.; Widyarani; Sintawardani, N. Utilization of tofu wastewater as a cultivation medium for Chlorella vulgaris and Arthrospira platensis. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *483*, 012027. [CrossRef]
149. Wall, R.; Ross, R.P.; Fitzgerald, G.F.; Stanton, C. Fatty acids from fish: The anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr. Rev.* **2010**, *68*, 280–289. [CrossRef] [PubMed]
150. Pereira, H.; Barreira, L.; Figueiredo, F.; Custódio, L.; Vizetto-Duarte, C.; Polo, C.; Resek, E.; Engelen, A.; Varela, J. Polyunsaturated Fatty Acids of Marine Macroalgae: Potential for Nutritional and Pharmaceutical Applications. *Mar. Drugs* **2012**, *10*, 1920–1935. [CrossRef] [PubMed]
151. Jung, I.S.; Lovitt, R.W. Integrated production of long chain polyunsaturated fatty acids (PUFA)-rich Schizochytrium biomass using a nutrient supplemented marine aquaculture wastewater. *Aquac. Eng.* **2010**, *43*, 51–61. [CrossRef]
152. Umamaheswari, J.; Shanthakumar, S. Efficacy of microalgae for industrial wastewater treatment: A review on operating conditions, treatment efficiency and biomass productivity. *Rev. Environ. Sci. Bio/Technol.* **2016**, *15*, 265–284. [CrossRef]