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Review

Beyond Bioremediation: The Untapped Potential of Microalgae in Wastewater Treatment

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Abstract: Microalgae-based wastewater bioremediation has emerged as a promising and sustainable solution for water purification by harnessing the natural ability of microalgae to absorb and transform pollutants. In the literature, it is possible to find diverse microalgae applications in wastewater treatment, highlighting their efficiency in nutrient removal, heavy metal sequestration, and overall water quality enhancement. Although microalgae demonstrate remarkable potential for wastewater treatment, there is a critical gap in research concerning the utilization of biomass produced during the treatment process, including large-scale biomass harvesting methods, economic viability assessments, and the exploration of innovative downstream applications. By shedding light on these deficiencies, the aim of this review is to encourage further research and development to maximize the potential of microalgae in removing wastewater pollution and the application of biomass derived from the treatment. In conclusion, this review not only underscores the overall efficiency of microalgae in wastewater bioremediation but also emphasizes the necessity of a more comprehensive approach that considers the full lifecycle of microalgae, from wastewater treatment to innovative applications of biomass, addressing both environmental and economic concerns.

Keywords: bioremediation; wastewater treatment; emerging pollutants; microalgae; biomass applications

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1. Introduction

Chemical water pollution has emerged as a significant concern and priority for society, public authorities, and especially the industrial sector. The sources of this pollution vary and include mining activities, industrial waste, urban wastewater (WW), sewage, pesticides and chemical fertilizers, energy usage, radioactive waste, and urban development [1]. Activities across the domestic, agricultural, and industrial sectors generate effluents containing harmful contaminants that expose human health and the environment to risks. Since these effluents are typically released into water bodies, it is crucial to continually protect water resources. Regulations governing the discharge of liquid industrial effluents are becoming increasingly stringent, particularly in developed countries, mandating that all wastewater should be treated before being discharged in nature [2]. The European Union Urban Wastewater Treatment Directive has been in effect for over 30 years, and since its adoption in 1991, there have been considerable improvements in the quality of European water bodies. European Union (EU) member states have established collection systems and wastewater treatment (WWT) facilities with the support of EU funding (https://environment.ec.europa.eu/topics/water/urban-wastewater_en; accessed on 15 July 2024). Currently, WW undergoes two main steps of treatment, namely primary and secondary treatment. The first is employed to remove large-size residues that could be present; the second one is mainly composed of aeration and settling tanks, where activated sludges are responsible for the removal of most of the pollutants. However, there are still issues with pollutants that are not covered by the current regulations. To address the latter, the Commission has proposed an update to the Directive [3]. Despite the progress made, traditional wastewater treatment methods are insufficient to completely remove many modern pollutants. Moreover, existing rules have not addressed pollution from smaller cities resulting from stormwater overflow. Paying attention to these challenges will be essential in order to achieve a pollution-free environment by 2050. More specifically, even though emerging pollutants (EPs) are frequently found in water bodies and are known to have dangerous effects on the environment, current legislation and treatment systems have not been able to deal with their proper removal from wastewater. Consequently, additional legislation and treatments to eliminate emerging contaminants before they enter the environment are urgently needed [4]. Microalgae are recognized as pollutant scavengers that are able to bioremediate a wide panel of chemicals released by several sectors, such as industrial, agricultural, and domestic. In addition to the common inorganic and organic compounds found in WW, such as heavy metals, xenobiotics, nitrates, phosphates, and carbon compounds, microalgal cells have the ability to uptake and decompose more persistent pollutants, such as antibiotics, polychlorinated biphenyls (PCBs), and hydrocarbons, which can be highly toxic to humans [5]. For instance, Zhou et al. [6] demonstrated that *C. pyrenoidosa* was capable of removing not only nitrogen and phosphorus from domestic WW, but also various heavy metals, such as mercury and silver (above 50%), as well as pharmaceuticals such as clarithromycin (around 80%). In addition, a microalgae-cyanobacteria consortium, namely *Chlorella vulgaris*, *Scenedesmus quadricauda,* and *Arthrospira platensis*, tested by Abdel-Razek et al. [7], was able to successfully remove malathion (up to 99%), a toxic pesticide, from urban WW. There are several papers in the literature demonstrating the efficiency of microalgae in removing excess of nutrients from water matrices; however, little is known about their ability to bioremediate emerging pollutants, either in pilot-scale systems or in real case scenarios [8]. In the context of a circular economy, this review aims to provide an updated overview of the release of major emergent pollutants, their already established treatments, which is the role of microalgae in these processes, and finally, to discuss what is missing to further implement microalgae-based treatment technologies.

2. Wastewater Composition and Conventional Treatment

The production of waste resulting from human activities is an inevitable fact of life, and a significant part of this waste consists of WW. The amount and quality of WW differs depending on several factors. In the case of households, the properties of their WW are influenced by residents' lifestyle, customs, socioeconomic status, and the societal environment in which they live [9]. By contrast, the WW composition of industries is affected by the processes used. WW is a multifaceted blend of various substances, and its composition can vary depending on its origin, treatment methods, and regional characteristics. A general list of the common types of WWs and the main pollutants present in each of them is reported (Table 1).

As can be seen from the diversity of compounds and the wide range of concentrations listed in Table 1, WW is a significant contributor to water pollution when it is not appropriately collected or treated in compliance with EU regulations. It contains elements like organic matter, nitrogen, and phosphorous compounds, which, when effectively treated, are removed to prevent eutrophication. Additionally, WW can become contaminated with harmful chemicals, bacteria, and viruses. If left untreated and released into the environment, these contaminants can negatively impact our health and harm our rivers, lakes, and coastal waters. Moreover, emerging pollutants in WW are a diverse group of contaminants that are gaining increasing attention due to their potential harm to both human health and aquatic ecosystems. These pollutants are characterized by their recent identification, increased detection in environmental media, and growing understanding of their potential impact. EPs often include substances that have not been widely recognized or studied in the past (see Chapter 3 for a more complete description). Advances in analytical techniques and increased awareness have led to the identification of new compounds in the environment.

Additionally, they exhibit persistence in the environment, resisting degradation over time, showing the potential to bioaccumulate in living organisms, and posing risks to higher trophic levels in ecosystems.

Table 1. Types of WW and main present pollutants.

Wastewater	Description	Main Pollutants		Amount	Ref.
Agricultural	Wastewater resulting from agricultural activities	Nutrients	Nitrogen	≈18–448 mg/L	[10–19]
			Phosphorus	≈5.4–7.1 mg/L	
		Pesticides and herbicides	Fluconazole, diazinon, diuron, atrazine, simazine, and malathion, metalochlor, tebuconazole tebuconazole and carbendazim	≈3 ng/L–27 µg/L	
		Organic matter	Chemical Oxygen Demand (COD)	≈1–30 g/L	
Dairy	Wastewater generated from dairy farms and processing facilities	Organic matter	COD	≈1–7.5 g/L	[20–24]
		Nutrients	Nitrogen	≈19.5–57.2 g/kg	
			Phosphorous	≈20–65.9 g/kg	
			Potassium	≈2.9–7.3 g/kg	
Domestic	Wastewater generated from households, including sewage and waterborne wastes from kitchens, bathrooms, and toilets	Biodegradable and non-biodegradable organic matter	Fibers	≈20.6%	[9,25–29]
			Sugars	≈10.7%	
			Proteins	≈12.4%	
		Soap and detergents	Alkylbenzene sulfonate	≈39 µg/L	
			Alcohol ethoxylate	≈6.2 µg/L	
			Alcohol ethoxy sulfate	≈6.5 µg/L	
			Alcohol sulfate	≈5.7 µg/L	
			Soap	≈174 µg/L	
		Pathogenic microorganisms	*Clostridium*, *Klebsiella*, *Corynebacterium*, *Bordetella*, *Staphylococcus* and *Rhodococcus*	≈1.5–5 log units	
		Pharmaceuticals and personal care products	Caffeine	≈61 µg/L	
			Ibuprofen	≈1.2 µg/L	
			Atenolol	≈2.9 µg/L	
			Triclocarban	≈2.4 µg/L	
		Suspended solids		≈120 mg/L	
Food processing	Wastewater from food processing plants	Organic matter	COD	≈1–9 g/L	[30–34]
			Biological Oxygen Demand (BOD)	≈9–20 g/L	

Table 1. *Cont.*

Wastewater	Description	Main Pollutants		Amount	Ref.
Industrial	Wastewater produced by industrial processes contains a variety of chemicals and contaminants specific to the industry	Oil and grease	≈50–66 mg/L	≈500 mg/kg	[35–43]
		Process and cooling water			
		Oils and grease		≈4–6000 mg/L	
		Organic matter	Total organic compound	≈100 and 3000 mg/L	
			Total naphthenic acids	≈113–392 µg/L	
			COD	≈22 g/L	
			BOD	≈10 g/L	
		Suspend solids		≈0.5–40 mg/L	
		Colorants and dyes		>1000 mg/L	
		Solvents	n-butanol dichloromethane, octanol, dimethyl formamide, and cyclohexanol	≈50–200 mg/L	
Hospital	Wastewater generated in healthcare facilities	Pathogenic microorganisms	*Acinetobacter* *Klebsiella* *Aeromonas* *Pseudomonas.*		[44–48]
		Chemical disinfectants	Povidone-iodine	≈0.4 ppm	
			Liquid chlorine Sodium hypochlorite Chlorine dioxide		
		Pharmaceuticals	Clozapine, chlorpromazine and risperidone	≈0.310–1432 µg/L	
		Antibiotics	Azithromycin, clarithromycin, and ciprofloxacin	≈21.2–4886 ng/L	
Leachate	Liquid that percolates through a landfill site, picking up contaminants from decomposing waste materials	Organic matter	COD	≈3.8–28 g/L	[49–51]
			BOD	≈1–11 g/L	
		Suspended solids		≈850–5840 mg/L	
		Toxic chemicals	Very different composition depending on which wastes are present in the landfill		
		Ammonia		≈1040–3560 mg/L	
Mining	Wastewater produced by mining operations	Heavy metals	Arsenic, Cadmium, Lead, Selenium, Iron and Zinc	≈200 g/L	[52–54]
		Acidic pH		<3.6	
		Suspended solids		≈22 mg/L	
		Dissolved solids		≈2900 mg/L	

Table 1. *Cont.*

Wastewater	Description	Main Pollutants		Amount	Ref.
Municipal/Urban	Mixture of domestic wastewater with industrial wastewater and/or runoff rainwater	Similar components to domestic wastewater with potential industrial additives			[55]
Paper and pulp mill	Wastewater from paper and pulp manufacturing facilities	Nutrients	Total phenol	≈39 mg/L	[56–58]
			Nitrogen	≈125–234 mg/L	
			Sulfate	≈1926–2098 mg/L	
		Organic matter	COD	≈17.9–19 g/L	
		Chlorinated compounds		≈3.12–5.43 mg/L	
		Suspend solids		≈80–90 mg/L	
		Dissolved solids		≈1 g/L	
		Solvents		≈0.4–3 g/L	
Stormwater	Rainwater runoff from precipitation events	Oil and grease		>4–800 mg/L	[39,59–62]
		Heavy metals	Lead	≈119–527 µg/g	
			Copper	≈50–464 µg/g	
			Zinc	≈241–1325 µg/g	
		Fertilizers and pesticides		≈0.1–10 ng/L	
Tannery	Wastewater produced by tanneries	Heavy metals	Chromium, Cadmium, Cobalt and Lead	≈500 mg/kg	[63–69]
		Organic matter	COD	≈1.3–2.6 kg/d	
		Suspended solids		≈1.2–28 g/L	
		High salinity		≈13.8 mg/L	
Textile	Wastewater from the textile and garment industry	Dyes and colorants		≈10–361 mg/L	[70–74]
		Heavy metals	Copper	≈0.0405 ppm	
			Lead	≈0.0003 ppm	
		Organic matter	COD	≈240 kg/d	
			BOD	≈60 kg/d	
		Suspended solids		≈100–336 mg/L	
		Dissolved solids		≈1.8–4.4 g/L	

Wastewater Treatment

Primary and secondary WWT are two essential stages in the process of cleaning and purifying domestic and industrial WW. Primary treatment is the first step, involving physical processes, such as sand filters and screening, to remove large debris, solids, and some organic matter. It effectively reduces the volume of pollutants in water but does not completely eliminate them, and it is important to note that primary treatment alone may not be sufficient to meet stringent water quality standards, especially in terms of removing dissolved pollutants, nutrients, and pathogens. Briefly, WW first enters the treatment facility and passes through screens or bars that catch large objects such as sticks, rags, plastics, and other debris [75]. The WW then enters a primary clarifier, namely a sedimentation tank or settling tank. Here, the flow velocity of the water is reduced significantly, allowing suspended solids and organic matter to settle to the bottom of the tank due to gravity. This forms a layer of sludge, while relatively clear water rises to the surface [76]. Any floating material, grease, or oil that accumulates at the surface of the sedimentation tank is skimmed off using mechanical skimmers or scrapers for further treatment or disposal. After that, the settled sludge at the bottom of the sedimentation tank is pumped out and sent to a sludge treatment process for further processing, such as dewatering and digestion. After primary treatment, WW undergoes secondary treatment processes in which biological and chemical methods are employed to further remove dissolved and suspended organic matter, as well as nutrients like nitrogen and phosphorus [77]. This may include the activated sludge process, which is a widely used method in which the primary treated WW is aerated and mixed with activated sludge, which is a suspension of microorganisms (bacteria, protozoa, and sometimes fungi) able to consume and break down organic pollutants in the WW [78]. Depending on the quality of the effluent required and the specific regulations governing discharge, after the primary and secondary treatment, WW might undergo tertiary treatment, such as filtration, disinfection (using chlorine, ultraviolet light, or ozone), or advanced oxidation to remove remaining contaminants and pathogens [79]. Certain organic contaminants may not respond well to chemical or conventional activated sludge methods of treatment. For instance, traditional activated sludge treatment effectiveness, studied in the French AMPERES research, showed an efficiency below 30% for 24 chemicals out of 97 [80]. As a result, water companies are adopting specialized treatment procedures to lower the concentration of emerging pollutants in wastewater treatment plant (WWTP) effluent water. The development of either additional or substitute advanced treatment processes, such as bioremediation through microalgae, could be a solution to further improve water quality, thus helping protect ecosystems and public health. Moreover, WWT is one of the biggest consumers of energy in the public sector; thus, the development of new and more sustainable techniques is mandatory.

3. Emergent Pollutants

"Emerging pollutants" is a broad term used to describe substances and groups of substances that have become the focus of scientific or political concern at specific times due to their environmental relevance. The definition by Farré et al. in 2008 from water analytics is as follows: "emerging pollutants are defined as compounds that are not currently covered by existing water quality regulations, have not been studied before, and are thought to be potential threats to environmental ecosystems and human health and safety' [81].

EPs are gaining attention due to their enduring nature, ability to accumulate in organisms, and resistance to typical WWT techniques. Even in small amounts, these contaminants can interfere with the organism's endocrine systems, damage aquatic life, and potentially infiltrate the food chain. The cumulative harmful effects on aquatic organisms and the environment are not yet fully comprehended, underscoring the importance of monitoring and addressing their existence and removal. In recent years, the presence of EPs like pharmaceuticals, endocrine disruptors, insecticides, and personal care products, has been detected in surface water, WW, and groundwater sources globally [82,83]. To mitigate these detrimental effects, various methods of EPs degradation and/or removal have been investigated. Numerous past and ongoing studies have concentrated on eliminating these contaminants using a range of treatment techniques, encompassing physical, chemical, and biological approaches. Innovative treatment approaches, including advanced oxidation processes and the utilization of natural biofilters like microalgae, are being investigated to diminish the presence of these contaminants in WW. The current European legislation relevant to emerging pollutants primarily falls under the framework of the European Union's water and chemical regulations. Key directives and regulations that address these pollutants include

1. Water Framework Directive (1997/0067(COD); WFD): a cornerstone of EU water policy aimed at protecting and restoring water bodies by preventing and reducing pollution. Emerging pollutants are a concern under the WFD as they can contaminate water bodies and affect aquatic ecosystems [84,85].

2. Priority Substances Directive (2000/60/EC PSD): identifies an index of priority compounds that need to be surveilled and controlled in European waters. Some emerging pollutants, like pharmaceuticals and personal care products, may be included in this list [86]. However, as our understanding of environmental risks evolves, new substances of concern are identified and added to the list if they meet certain criteria regarding their persistence, bioaccumulation, toxicity, and potential for widespread environmental exposure. Once included in the list, these substances are subjected to monitoring, assessment, and control measures to mitigate their impacts on water quality and safeguard ecosystems and public health.
3. Pharmaceuticals in the Environment (EMEA/CHMP/SWP/4447/00): The European Medicines Agency (EMA) has issued guidelines on the environmental risk assessment of pharmaceuticals, ensuring that pharmaceutical companies consider the environmental impact of their products [87].
4. Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH Regulation; 1999/45/EC) addresses the registration, evaluation, and authorization of chemicals. It plays a role in managing and controlling industrial chemicals, some of which may be considered EPs [88].
5. Biocidal Products Regulation (528/2012/EU; BPR): regulates the use of biocidal products, which include chemicals used for disinfection and pest control. Some of these products may be considered EPs when they find their way into the environment [89].
6. Wastewater Treatment and Discharge Regulations (91/271/EEC): European nations have specific regulations for WWT and discharge. These regulations often include standards for the removal of EPs, particularly in urban areas [3].
7. European Pharmacopoeia: This reference work contains quality standards for medicines in Europe and includes guidelines on the environmental risk assessment of pharmaceuticals [90].

Although these existing regulations provide a framework for addressing EPs, there are still some challenges that need to be addressed. On the one hand, monitoring and risk assessment are the most valuable ways to set up new technologies and fill regulatory gaps. Nevertheless, policymakers must continually update and expand regulations to keep pace with the evolving scientific knowledge.

It is important to point out that the presence and impact of these EPs can vary widely depending on their specific chemical properties and how they are used and disposed. Additionally, the list of EPs continues to evolve as new compounds are identified and studied, making ongoing research and regulatory efforts essential to effectively address this environmental and public health challenge. The main categories of EPs are reported in Table S1 (Supplementary Material); however, the list is expanding daily, posing a multifaceted challenge for European regulators and researchers. Current European legislation provides a foundation for addressing these contaminants; however, ongoing efforts are required to enhance monitoring, risk assessment, and regulation to ensure the protection of the environment and human health in the face of a continuously evolving array of emerging pollutants [91,92].

4. Microalgae as a Promising Solution

Reducing climate change, using natural resources sustainably, and lowering environmental pollution are today's major global challenges. Global food, water, and energy demands are predicted to increase by 60%, 80%, and 50%, respectively, between 2000 and 2050 [93,94]. One logical approach to reducing water consumption and demand could be to integrate WWT with algal cultivation, as both industries operate on comparable scales and utilize similar production facilities; thus, the application of microalgae for WWT could be a valuable solution among the others [95,96]. WWT systems utilizing microalgae offer inherent disinfection capabilities and surpass traditional WWT approaches in mitigating nutrient pollution. Unlike conventional methods with drawbacks like elevated operational expenses and inevitable secondary pollution from chemical procedures, microalgae stand out as highly effective decontaminating agents [97]. Their notable surface-to-volume ratios result in impressive biosorption capacities, allowing them to eliminate harmful substances. Additionally, many microalgae species exhibit versatility by transitioning between photoautotrophic, mixotrophic, and heterotrophic growth modes [98]. Compared to exclusively heterotrophic microorganisms, the main benefit of utilizing microalgae is that their growth is not restricted by decreasing pollution concentrations. The growth rate of microalgae may vary depending on different factors, such as species, nitrogen and phosphorus concentrations, and light intensity [99]; however, their adaptability represents an additional resource for this application.

4.1. How Do Microalgae Work?

The removal of contaminants by microalgae can occur in different ways, such as bioconversion, bioadsorption, and bioaccumulation [100].

4.1.1. Bioconversion

One highly effective method for removing pollutants from wastewater is bioconversion, wherein composite compounds are broken down into simple, harmless chemical building blocks that can be used as carbon sources. Unlike biosorption and bioaccumulation, in which microorganisms act as biological filters to separate and concentrate pollutants from water, biodegradation involves the decomposition of target pollutants. This can occur through biotransformation, which is a sequence of enzymatic events that result in different metabolic intermediates, or through full mineralization of the parent molecules into CO_2 and H_2O [101,102]. The fundamental mechanisms of biodegradation fall into two groups: (i) metabolic degradation, in which pollutants act as electron donors/acceptors and carbon sources for microalgae and (ii) cometabolism, where pollutants serve as both electron donors and carbon sources [103]. Biodegradation mediated by microalgae can occur in the extracellular or intracellular environment or through both. Enzymes present in microalgae, or released into the medium by them, such as peroxidases, azo-reductase, and laccases reported by several authors [101,102,104,105], are able to break down these complex molecules into simpler molecules to be used as metabolites by different mechanisms. Extracellular degradation involves the excretion of enzymes by microalgae, which break down pollutants outside the cell walls, enabling the mineralization of dissolved pollutants and functioning like an external digestive system. Additionally, this process may lead to an increase in the bioavailability of pollutants in the environment, hence facilitating microalgae following bioaccumulation. Three stages of intricate enzymatic reactions are involved in the microalgae-based biodegradation of organic contaminants. Stage I employs cytochrome P450 enzymes, including hydroxylase, carboxylase, decarboxylase, and monooxygenase, which enhance the hydrophilicity of the pollutant by adding or revealing a hydroxyl group through oxidation-reduction reactions or hydrolysis [101,106]. In stage II, enzymes such as glutathione-S-transferases and glucosyltransferases facilitate the conjugation of glutathione with various compounds possessing electrophilic centers to protect the cell from oxidative damage [107]. The third stage of detoxification involves a range of enzymes, including oxidoreductases, such as glutamyl-tRNA reductase, carboxylases, mono(di)oxygenases, laccases, transferases, hydrolases, pyrophosphatases, and dehydratases, which convert compounds into intermediates that are either less harmful or non-toxic [104]. In contrast, cometabolism involves the use of pollutants as both electron donors and carbon sources. These organic substrates act as electron donors, aiding in the co-metabolization of EPs and biomass [108]. To enhance microalgae-mediated biodegradation of persistent pollutants, some studies have suggested adding nutrient substances or organic substrates to create a co-metabolic system [109]. Vo et al. [109] demonstrated that adding glucose to *Chlorella* sp. cultures increased the removal efficiency of all EPs tested, namely, tetracycline, sulfamethoxazole, and bisphenol A, above 80%. Although research indicates that the highest concentrations of detoxifying enzymes and the best removal effectiveness come from employing sugar-carbon sources, adopting some of them may decrease the removal efficiency of EPs, most likely as a result of catabolite suppression [108]. Therefore, it is essential to evaluate different carbon sources to identify the most suitable ones for effective pollutant biodegradation in wastewater.

4.1.2. Bioadsorption

Bioadsorption involves the adsorption of compounds (sorbates) by microalgae onto the cell membrane (solid-phase sorbent) [110]. While bioconversion can only be carried out by living cells, bioadsorption can be carried out by either living or dead cells [111]. Biosorption involves physical, chemical, and metabolic-independent processes facilitated by mechanisms such as precipitation, ion exchange, surface complexation, and electrostatic interactions [112]. Microalgae contain functional groups on their membranes that make them negatively charged, attracting metal ions (usually dissolved in the form of cations) via electrostatic forces. The large surface area of small-sized cells in large quantities in water aids the removal process [113,114]. Furthermore, microalgae contribute to the organic matter suspended in water, which is capable of adsorbing other organic compounds, such as dyes or other organic pollutants. The high affinity of the biosorbent for the target sorbate drives the attraction of the sorbate molecules [110,111]. The process continues until equilibrium is reached between the sorbate adsorbed by the biosorbent and its remaining concentration in the liquid. Moreover, the porosity and roughness of the microalgae surface affect heavy metal adsorption [115]. Trace elements like manganese, molybdenum, cobalt, copper, zinc, iron, and boron are essential for enzymatic processes and cell metabolism but can be toxic at high concentrations. In contrast, heavy metals such as chromium, cadmium, mercury, lead, and arsenic are generally toxic to microalgae [116]. Flocculation can be promoted by active binding sites on the cell surface, forming complexes with specific pollutants and reducing total dissolved and suspended solids [4]. Mota et al. [117] discovered that polymeric material released by *Cyanothece* sp. can sequester heavy metals from contaminated water due to carboxyl and hydroxyl functional groups rather than ion exchange.

4.1.3. Bioaccumulation

Microalgae can accumulate various pollutants along with nutrients and microelements [118]. Differently from biosorption, bioaccumulation requires energy since it is an active metabolic process, resulting in a relatively slow system [119]. This process is crucial for removing inorganic and organic pollutants (such as sulfates, nitrates, phosphates, heavy metals, and pesticides) as these substances are transferred into the cells [110]. Additionally, changes in cell membrane permeability due to pollutants or environmental stress can result in passive diffusion mediated by membrane depolarization or hyperpolarization [120]. Although bioaccumulation and biosorption are fundamentally different, quantifying the pollutants processed by each mechanism is challenging because the two mechanisms are dynamically interrelated. Previous studies have reported the bioaccumulation of sulfamethoxazole, trimethoprim, florfenicol, and carbamazepine, where these pollutants enter microalgal cells through passive diffusion [121]. For instance, the cyanobacterium Anabaena CPB4337 showed altered sensitivity to herbicides when exposed to perfluorinated alkyl acid compounds like perfluorooctane sulfonate and perfluorooctanoic acid, with some herbicides becoming more toxic and others less toxic [122]. Moreover, Xiong et al. [102] demonstrated that the bioaccumulation of levofloxacin by Chlorella vulgaris significantly increased from 34 to 101 μg/g with the addition of 1% (*w*/*v*) NaCl.

In addition, microalgae can help reduce greenhouse gas (GHG) emissions by aiding in the sequestration of carbon dioxide (CO_2) from the atmosphere or from industrial flue gases. Moreover, wastewater's aquatic environment provides an ideal environment for microalgae to grow. The synergistic relationship between microalgae and bacteria provides additional opportunities and benefits in WWT [123], enhancing the overall efficiency and effectiveness of the WWT processes. Microalgae have the ability to assimilate nitrogen and phosphorus compounds from WW through photosynthesis [124]. Bacteria can further transform nitrogen compounds, such as ammonia and nitrate, into nitrogen gas through biological processes like nitrification and denitrification [125]. This combined action of microalgae and bacteria promotes the efficient removal of nutrients from WW, reducing the risk of eutrophication in receiving water bodies. Microalgae produce oxygen as a byproduct of photosynthesis, which increases the dissolved oxygen levels in WW. This oxygenation is beneficial for aerobic bacteria, which require oxygen to effectively metabolize and degrade organic pollutants [126,127]. Oxygen aeration represents more than 50% of the energetic needs of a WWTP; thus, from an economical point of view, the synergistic use of microalgae and bacteria can reduce the overall cost of the process [128]. The efficiency of the algal-bioremediation processes discussed earlier is influenced by a range of physico-chemical factors, including pH, redox potential, temperature, duration and strength of light exposure, hydraulic retention time, size of adsorbents, and concentration of environmental pollutants [129].

4.1.4. Temperature, pH, and Hydraulic Retention Time

Industrially generated WW effluents often exhibit elevated temperatures, posing the risk of thermal pollution in aquatic ecosystems [130]. Although different microalgae species have specific temperature requirements, they can generally thrive within a broad temperature range. The optimal temperature for commonly cultivated microalgae typically falls between 15 and 28 °C [131]. Once this optimal temperature is surpassed, there is a noticeable decline in biomass productivity. Temperature fluctuations pose a significant challenge in the cultivation of microalgae. Hence, microalgae species capable of thriving in high-temperature WW environments (around 30–40 °C) are crucial for effectively utilizing these organisms in the bioremediation of emerging pollutants [132].

pH serves as a critical factor influencing the effectiveness of algal-bioremediation mechanisms, playing a role in altering the ionization states of various functional groups present on the surface of microalgae [133]. Any deviation from the optimal pH level for a biological process can reduce the reaction rate. This occurs because the charge of the cell membrane of microalgae is positive owing to lower pH levels, leading to decreased molecule adsorption. Conversely, when the pH surpasses the isoelectric point, the algal surface acquires a negative charge, resulting in enhanced absorption [134]. Specifically, pH levels exceeding 9 have a detrimental effect on algal growth, as they significantly reduce the capacity for carbon dioxide absorption and hinder the activity of RuBisCO, decreasing photosynthetic activity [135].

The hydraulic retention time (HRT) can influence certain mechanisms by which microalgae uptake pollutants. In certain instances, a shorter hydraulic retention time may result in partial or ineffective elimination of pollutants. The efficacy of pollutant removal depends on both the biomass of microalgae present and the hydraulic retention time since a very short HRT could lead to washout of the culture [136]. Removal rates tend to increase with higher concentrations of microalgae and longer hydraulic retention times up to a certain limit [137].

4.1.5. Particle Size and Adsorbent Dose

The permeability for absorption into the cell wall improves as the pollutant particle size decreases, and this interaction is affected by the pollutant's toxicity. This effect is due to the increased surface area, which provides more binding sites. The functional groups engaged in the interaction, electrostatic capacity, and surface area are some of the factors that affect algal bioremediation by adsorption. Moreover, longer hydraulic retention durations between the contaminants and the adsorbent have been shown to boost absorption [138,139].

4.1.6. Light Exposure and Redox Potential

Photolysis and hydrolysis help reduce the persistence and toxicity of pollutants in the environment and are two key processes that break down pollutants in the environment. The first is the breakdown of pollutants due to exposure to sunlight, particularly to ultraviolet (UV) radiation. The latter causes the chemical bonds within the pollutant molecules to break, leading to their degradation into smaller molecules. The second mechanism consists of the chemical breakdown of pollutants by water. Water molecules react with the pollutant, leading to the cleavage of chemical bonds, resulting in the formation of new, often less toxic, compounds [140,141].

The efficiency of microalgae in bioremediation is influenced by a range of biological, nutrient, and environmental factors in addition to the abovementioned factors [142]. Biological factors include the specific algal species used, as different species have varying capacities for pollutant uptake and degradation [143]. The growth phase of the algae also impacts its bioremediation potential, with different stages of growth showing different levels of activity. Furthermore, interactions with other microorganisms such as bacteria and fungi can either enhance or inhibit algal performance [144]. Nutrient availability is another crucial factor; the composition and availability of essential nutrients like nitrogen, phosphorus, and micronutrients are vital for optimal algal growth and pollutant removal. Conversely, nutrient limitation can significantly hinder these processes [101]. Environmental factors such as salinity levels and the presence of inhibitors like toxic substances, can also affect algal growth and bioremediation efficiency, as high salinity or toxic inhibitors can suppress algal activity and pollutant uptake [145]. While axenic (pure) microalgae cultures can offer more controlled conditions and higher efficiency in pollutant removal, they present significant economic drawbacks. Maintaining axenic cultures is costly and labor-intensive, requiring sterile conditions and specialized equipment to prevent contamination [146]. This increases the overall cost of the bioremediation process, making it less economically viable for large-scale applications. Additionally, the need for continuous monitoring and the risk of culture collapse further adds to operational expenses, limiting the practicality of using axenic microalgae cultures in real-world scenarios. Understanding these factors is essential for optimizing microalgae-based bioremediation systems while balancing their efficiency and economic feasibility.

4.2. Microalgae-Based Wastewater Treatment

In recent times, there has been a growing concern regarding the presence of PAHs, PCBs, pesticides, pharmaceuticals, and heavy metals, among other pollutants in water. In Europe, the Water Framework Directive (Directive 2000/60/EC) does not mandate that WWTPs address these micropollutants. However, it does specify a list of substances that member states need to monitor. Additionally, the REACH regulation (2006) bolstered EU regulations by requiring a risk assessment of 30,000 chemical substances. The European Union IPPC (Directive 2008/1/CE) and subsequently the Industrial Emission Related Directive (Directive 2010/75/UE) have replaced the Water Framework Directive. These directives call for the application of the best available techniques (BAT), considering both technical and economic preventive measures, pollution control technologies, and efficient resource consumption. Given the bioremediation potential of microalgae, there is a specific opportunity to innovate and implement new technologies for WW bioremediation based on microalgae. Currently, several research groups are studying the use, mechanisms, and efficiencies of a wide variety of microalgae strains and consortia for WWT (Table 2).

Table 2. Studies of microalgae-based treatments for the removal of pollutants from WWs and subsequent application of the produced biomass.

Strains	Type of WW	Volume Treated (L)	Exp Time (Day)	Pollutants	Removal Rate	Biomass Application	Ref.
Consortium of *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Arthrospira platensis*	Urban WW	10	28	Malathion	99%	/	[7]
	Agricultural drainage			Cadmium	88%		
	Combination of both			Nickel	95%		
				Lead	89%		
Galdieria phlegrea	Urban WW	1	9	Ammonium	≈70%	/	[147]
				Phosphates	≈22%		
Co-culture of *Chlorella vulgaris* and *Arthrospira platensis*	Winery WW	0.2	15	Polyphenols	50%	Biofuel	[148]
Scenedesmus sp.	Tannery WW	0.25	12	Chromium	up to 96%	/	[149]
				Copper	up to 98%		
				Zinc	up to 98%		
				Lead	up to 98%		
Scenedesmus quadricauda and *Tetraselmis suecica*	Dairy WW	10	12	Nitrates	≈95%	Tetracycline bioadsorption ≈ 70%	[150]
				Phosphates	≈89%		
				Sulfate	100%		
Chlorella pyrenoidosa, *Chlamydomonas reinhardtii*, *Scenedesmus obliquus* and *Chlorella vulgaris*	Domestic WW	1	7	Mercury	>50%	/	[6]
				Lead	>50%		
				Gold	>50%		
				Silver	>50%		
				Manganese	>50%		
				Clarithromycin	80%		
				Roxithromycin	>50%		
				Triclocarban	>50%		
Chlorella pyrenoidosa	Textile WW	1	7	Indigo dye	89%	/	[119]
				Direct blue dye	79%		
				Remazol brilliant orange	75%		
				Crystal violet dye	72%		
Arthrospira platensis and *Arthrospira maxima*	Mining WW	0.5	12	Sulfate	73%	/	[151]
				Nitrogen	up to 86%		
				Phopshorous	up to 80%		
C. vulgaris	Urban WW	4.5	9	Siloxane	≈98%	/	[152]
Leptolyngbya sp. and *Chroococcus*	Brewery WW	1	15	Nitrates	67%	Bioethanol production	[153]
				Ammonium	98%		
				Total Phosphorous	75%		

Table 2. *Cont.*

Strains	Type of WW	Volume Treated (L)	Exp Time (Day)	Pollutants	Removal Rate	Biomass Application	Ref.
Natural Bloom	Domestic WW	60	30	Ammonium	55%	/	[154]
				Total Phosphorous	91%		
Chlorella vulgaris and *Scenedesmus almeriensis*	Synthetic WW	0.5	3	Arsenic	up to 35%	/	[123]
				Copper	up to 98%		
				Manganese	up to 78%		
				Zinc	up to 83%		
Enterobacter sp. MN17 and *Chlorella vulgaris*	Textile WW	5	5	Cadmium	93%	Bioenergy production	[155]
				Chromium	79%		
				Copper	72%		
				Lead	79%		
Nannochloropsis gaditana, *Chlorella sorokiniana*, *Chlorella* sp. and *Dunaliella tertiolecta*	Municipal WW	0.1	15	Total Nitrogen	77%	Biostimulants, biofuels, bioplastics, Syngas	[156]
				Total Phosphorous	61%		
Arthrospira platensis	Industrial WW	30	8	Copper	68%	Bioethanol	[157]
				Nickel	75%		
				Zinc	42%		
				Chromium	24%		
Nannochloropsis sp.	Synthetic WW	2	6	Paracetamol	≈50%	Biobased feedstock	[158]
				Ibuprofen	≈50%		
				Olanzapine	≈50%		
				Simvastatin	≈50%		

As shown in Table 2, microalgae can effectively remove nutrients from wastewater. Di Cicco et al. demonstrated that *Galdieria phlegrea*, an extremophilic microalga, removed about 70% and 22% of ammonium and phosphates, respectively, from urban WW [147]. Daneshvar's research team also studied the removal of nutrients from municipal wastewater using *Tetraselmis suecica* and *Scenedesmus quadricauda*. The results showed that *S. quadricauda* outperformed *T. suecica*, achieving removal efficiencies of 95.3% for nitrates and 89.8% for phosphates [150]. As nutrient removal with microalgae is an already established reality, some researchers have shifted their attention to the clean-up of EPs from WW. For instance, Zhou et al. [6] reported that some microalgae species, including *C. reinhardtii* and *C. pyrenoidosa*, were able to remove a wide variety of heavy metals, such as silver and mercury, and pharmaceuticals, such as clarithromycin, with removal efficiencies above 50% and above 80%, respectively, from domestic WW. Ajayan et al. also reported high removal rates of heavy metals, including lead, by *Scenedesmus* sp., cultivated in tannery WW. After 12 days of growth, *Scenedesmus* sp. removed between 75% and 98% of lead [149]. Similar removal efficacies for this metal were also reported by Abdel-Razek et al. for both urban and agricultural WW samples using a consortium of *Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Arthrospira platensis* [7]. Moreover, this designed consortium of microalgae and cyanobacteria was able to successfully remove malathion (up to 99%), a toxic pesticide, from the same WW samples [7]. A similar consortium of *Chlorella vulgaris* and *Arthrospira platensis* was reported to be able to remove polyphenols from winery WW with removal yields above 50% [148]. Moreover, two species of the genus *Chlorella*, *C. vulgaris* and *C. pyrenoidosa*, have been documented by Premaratne et al. to have high decolorizing power for various types of dye: "Indigo" (89.3%), "Direct blue" (79%), "Remazol brilliant orange" (75.3%) and "Crystal violet" (72.5%). During this process, the COD of the WW was diminished by 70% [100,159]. Moreover, Saavedra et al. investigated the influence of organic matter or CO_2 on heavy metal removal from synthetic WW using *Chlorella vulgaris* and *Scenedesmus almeriensis* [123]. Noteworthy is the fact that most of the results reported in Table 2 were obtained at a laboratory scale, indoors, with the use of artificial light (ranging from 45 to 100 µmol/m·s) and, most importantly, of low working volumes, with 10 L being the highest tested volume. In addition, given the small-scale experiments, no information on the hydraulic retention time, one of the most major parameters, is provided. In Table 2, only two research works are reported that have been carried out with larger volumes; Sayara et al. evaluated the role of the natural consortium of microalgae and cyanobacteria in scavenging ammonium and phosphorus (55% and 91%, respectively) from domestic WW [154], while, Serrà et al. demonstrated the ability of *Arthrospira platensis* to remove heavy metals from industrial WW. However, only the latter applied the concept of a more circular approach, as the results suggested that biomass could be used in the production of bioethanol [157]. For this reason, industrially relevant studies with larger volumes under real atmospheric conditions and with real WW are required before considering the large-scale application of microalgae in the treatment of WW. On top of that, most of the papers mentioned in Table 2 describe the rates of pollutant removal by the microalgae-based treatment; however, none of them focus on the contamination level of the biomass itself. It is important to keep in mind that using WWs as a culture medium may lead to differences in microalgae biomass, such as higher lipid or protein content. Moreover, it is worth evaluating the degree of contamination of the biomass and quantifying the pollutants that have been taken up/sequestered by the biomass from the medium.

Drawbacks

From an industrial and commercial point of view, microalgae are typically grown in artificial nutrient solutions, which adds a significant expense to the product. Nonetheless, this cost fluctuates depending on the specific species employed, and it can be mitigated by using waste materials as a source of nutrients. Chisti et al. [160] assessed the expenses associated with establishing *Arthrospira platensis* biomass production facilities, while Fernández et al. [161] conducted an in-depth analysis on a case study involving a 5-hectare facility utilizing open reactors, raceway, and thin-layer cascade systems. The results confirm that the production costs of microalgae biomass for these applications are 4.5 and 2.3 €/kg when employing the raceway and thin-layer cascade, respectively. To achieve cost reduction, it is essential to utilize WWs as a nutrient source, leading to biomass production costs of 3.6 and 1.4 €/kg for the raceway and thin-layer cascade, respectively. Further cost reduction necessitates a significant decrease in the manpower required for the process operation. By reducing up to 0.1 person/ha, the biomass production cost can be lowered to 2.6 and 0.8 €/kg for raceway and thin-layer cascade production systems, respectively. When considering the process as a WWT method, the treatment cost can be minimized to 1.1 and 1.2 €/m^3 for raceway and thin-layer cascade, respectively. These costs are still higher than those associated with conventional WWT systems, which stand at 0.2 €/m^3. However, if the WWT cost is included as an input for biomass production, the final biomass production cost can be reduced to minimum values of 2.1 and 0.6 €/kg using the raceway and thin-layer cascade, respectively. Thus, replacing synthetic media with industrial effluents emerges as a crucial alternative for cost reduction in microalgae production, simultaneously serving as a means to treat effluents, rather than relying on conventional physical, chemical, and biological methods [162]. WWs exhibit nitrogen and phosphorus concentrations up to three times higher than those found in natural water bodies [163,164], and microalgae rely on these nutrients for energy exchange and growth, reducing the overall eutrophication potential of WWs [165]. Consequently, these microorganisms are able to grow sufficiently, offering a high number of cells that can contribute to effluent bioremediation by removing pollutants from the water or even breaking down contaminants into metabolites that are less harmful than the parent compounds [166]. To address the global challenges of water scarcity and the non-renewability of phosphorus [167], the rational use of nutrients and water is vital. Valorizing biomass after WWT is crucial from a circular economy perspective. In this sustainable approach, the by-products of WWT, which are rich in organic matter, are not treated as mere waste but as valuable resources. The biomass obtained from WW can be harnessed to produce biogas, biofuels, or even biobased chemicals. Indeed, the most important aspect for governments and policymakers is the quality of water at the end of the process. However, depending on the abovementioned factors, biomass can find different applications that generate revenue, help mitigate the costs of the overall processes, and increase the circularity of WWT. By integrating these biomass valorization techniques into the circular economy model, the loop of resource usage can be closed, promoting a regenerative system where waste becomes a feedstock for new products. This not only minimizes the environmental impact but also contributes to energy generation and the development of a biobased economy, fostering a more sustainable and resilient future.

5. Biomass Utilization

In addition to their capacity for carbon capture, microalgae are a rich source of diverse molecules, with potential applications spanning from biofuel production [168,169] to industries such as cosmeceuticals and nutraceuticals. Given the recognized long-term drawbacks associated with synthetic compounds, there is an urgent need to explore new natural substances possessing beneficial properties [170], making microalgae a promising alternative. For instance, dried biomass can be repurposed as animal feed [171] or fertilizer [172], and the carbohydrates present in microalgae can be utilized for lactic acid production [173,174] or for bioplastic production [173,175]. Depending on the predominant microalgae strain, the dried wastewater-grown biomass can also be used as a feedstock for valuable compounds. For example, phycocyanin can be obtained from cyanobacteria [176], whereas other microalgae may be a source of high-value molecules like omega-3 [177] or carotenoids [178], which are often used as natural colorants in the food sector because of their remarkable color, antioxidant, and preservative properties [179]; however, the applicability of wastewater-grown microalgal biomass may be limited due to specific optimization process requirements that affect the productivity of such compounds, and strict industry regulations that prevent the use of biomass produced in WW. Therefore, it is crucial for microalgae biomass intended for such applications to be free of persistent pollutants like heavy metals, which could adversely affect animals, soil, or groundwater. The transition from lab- to industrial-scale processes often involves several challenges, engineering, chemistry, economics, and regulatory compliance, which can result in loss of productivity. Addressing these challenges requires careful planning, experimentation, and continuous optimization to ensure a smooth transition and to maintain or improve productivity on a larger scale.

The most promising application of this biomass appears to be in the energy market, where drying can be avoided [180]. A considerable number of studies have highlighted the essential role of WW in advancing the microalgae biofuel production industry [181–184]. Presently, microalgae production is expensive, leading to costs as high as 100 €/kg of biomass. For instance, a 30 m^3 tubular photobioreactor plant incurred a production cost of 69 €/kg of dry weight over two years of continuous operation [184]. Moreover, the differences between these costs and the aforementioned costs are mainly due to the production system adopted. Closed and open bioreactors primarily differ in operational complexity, environmental control, and resource requirements. For instance, Oostlander et al. [185] carried out techno-economic analyses on the operational costs of producing microalgae in tubular-shaped reactors and airlifts, both under artificial light, in the Netherlands, determining a cost of 290 €/kg and 587 €/kg, respectively. The reported costs in the literature may vary widely because of the location and system of the production of microalgae. In summary, open bioreactors have lower initial and operating costs but may be less productive and more prone to environmental disruptions, while closed bioreactors, though more expensive, offer higher yields and better control over production conditions. Only through techno-economic projections is it possible to estimate the costs for large-scale production, but outcomes vary based on assumptions and key parameter values like lipid productivity.

In Table 2, different applications of the biomass obtained with diverse WWs as culture medium are shown. Several authors have studied the efficiency of microalgae in WWT; nevertheless, only a few of them have focused on the further utilization of the biomass obtained through these cultivations. Papadopoulos et al. demonstrated the ability of a cyanobacteria consortium to remove heavy metals from electrochemically pretreated brewery WW and to obtain carbohydrate-enriched biomass for bioethanol production [153]. Lima and colleagues confirmed that the lipid content of biomass can vary depending on cultivation conditions. They evaluated the total nitrogen and total phosphorous removal from municipal WW with different indigenous strains from Sicily, resulting in biomass fitting for biofuel production [156].

Following biomass harvesting and water removal, the dry weight concentration typically ranges from 15% to 25% [186]. For example, it is possible to extract lipids by procedures applied to the wet biomass, converting it into energy [187], such as anaerobic digestion or hydrothermal liquefaction (HTL), and then converting them via transesterification [188]. Recent screenings have identified strains that grow on WW and accumulate lipids simultaneously (up to 23.7 mg/L/day) [189]. Anaerobic digestion efficiently converts microalgae biomass into biogas but currently lacks economic viability. Competing with the globally low price of natural gas poses a challenge for biogas obtained by anaerobic digestion. Furthermore, the expense associated with anaerobic digestion exhibits considerable variability across different feedstocks, primarily because of economies of scale [190]. HTL is a thermochemical process, and its main advantage is the direct use of wet biomass as feedstock, reducing the overall energy demand, eliminating the drying stage of the biomass [191], and transforming wet biomass into bio-crude, gas, residual solids, and an aqueous phase rich in nutrients. Although several papers have recently been published in the literature, there are still gaps in the process for a large-scale application, such as corrosion in reactor tubes, salt deposition, product stability, heat recovery, and emulsion [191].

In order to improve the sustainability of the process and decrease the overall production costs, recycling of cultivation media has been explored [192–194]. Despite these efforts, the petroleum diesel cost (0.6 €/L) still represents an unreachable point for microalgae-based biodiesel, mainly due to the estimated production cost (2.5 €/L) [195]. Microalgal biomass obtained from cultivation on WW may not always meet safety regulations due to its chemical and biological composition, fouling by pathogenic organisms, or toxic pollutants. In such cases, converting low-grade biomass into biochar through pyrolysis emerges as a valuable alternative. Depending on the biochar composition, it can be utilized as a soil amendment [196], minimizing the risk of leaching toxic materials like heavy metals, as the pyrolysis process captures these metals in the solid matrix [197].

6. Final Considerations

Microalgae biomass possesses numerous potential uses, with the most promising large-scale application being adopted as biofuels [198]. Additionally, they have already established roles in high-value markets such as human dietary supplements (nutraceuticals) and animal feed [199]. However, achieving economic, energetic, and, above all, environmental sustainability in microalgae production requires significant advancements [180]. One promising opportunity for microalgae is their utilization in WWT. Wastewaters are rich in nitrogen and phosphorus, which are essential nutrients for microalgal growth [163,200]. Thus, microalgae can help remediate effluents by converting contaminants into nutrients and less toxic substances [166]. This approach, known as a microalgae-based biorefinery coupled with biochemical effluent treatment, is gaining interest in the scientific community. Commercially, microalgae are typically grown in synthetic media, which can be expensive. However, the cost varies depending on the scale and the specific species used, and it can be reduced by using effluents from industrial processes. Several studies have demonstrated that the culture medium can account for a significant portion of the total production costs [160,201]. Moreover, the work from Feng et al. [202] demonstrated that growing *Chlorella vulgaris* in artificial effluent can reduce the cost of 1 ton of biomass by up to 3.5 times (from 750 € to 215 €). Substituting synthetic media with industrial effluents not only reduces costs, but also provides a means of treating these effluents, which is more environmentally friendly than traditional methods. This approach not only offers a cost-effective means of cultivation but also a new potential market segment. Microalgae are able to efficiently uptake a wide range of chemicals from water [203], regardless of their various nutritional modes (phototrophy, heterotrophy, and mixotrophy), conferring these microorganisms with high versatility and potential. Among the strategies for large-scale microalgal biomass production, combining WWT with algal farming makes sense owing to the similar scale and production facilities shared by both industries. An additional benefit of this approach is the improvement of local industries and the reduction in the environmental impact related to nutrient manufacturing, transportation, and changes in land use. It is crucial to conduct economic feasibility assessments for WWT systems based on microalgae and compare them to traditional methods, given the limited number of existing studies on pilot or industrial-scale production. Meanwhile, the absence of fundamental design and operational guidelines for microalgae-based WWT should serve as a driving force for researchers to intensify their efforts. Their aim should be to enhance the adaptability and robustness of microalgal strains in addressing diverse types of WW challenges to provide essential guidance and recommendations [165]. Moreover, improving the Technology Readiness Level (TRL) 3 to TRL 9 in a microalgae-based WWT is crucial for transitioning from laboratory research to full-scale commercial application. At TRL 3, the technology is still in the experimental proof-of-concept stage, where fundamental principles are being validated in controlled environments. Advancing through TRLs involves progressively refining the technology, optimizing processes, and scaling up systems to address real-world challenges, such as nutrient removal efficiency, cost-effectiveness, and environmental impact [204]. This progress is vital for meeting environmental regulations, reducing treatment costs, and contributing toward achieving circular economy objectives. The continuous development and discovery of new technologies in this field extimates the global microalgae market, reaching 1.143 USD billion by 2024, growing at a compound annual rate of 7.4% [205].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16192710/s1, Table S1. Examples of EPs divided by major groups.

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