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Review

Review and Opinions on the Research, Development and Application of Microalgae Culture Technologies for Resource Recovery from Wastewater

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Abstract: Wastewater is an abundant source of nutrients and energy. Under the circumstances of circular economy and carbon neutrality, resource recovery from wastewater has recently been motivated. The microalgal process is a promising alternative for resource recovery from wastewater, and it possesses potential for the cost reduction of wastewater treatment and fertilizer production, energy generation, and greenhouse gas emissions reduction. This paper reviews and discusses state-of-the-art microalgal process development, including microalgal species screening, configuration, biotic consortia construction, infection avoidance, nutrients balancing, operational parameter optimization, and biomass harvesting enhancement. Due to the lack of literature on practical applications, the microalgal process lacks economic and environmental feasibility assessments. Life cycle assessments from the perspective of circular economy and carbon neutrality on upscaled microalgal processes are required for various wastewater scenarios. To promote the upscaled application and successful implementation, efforts are also suggested to establish utilization guidelines, advanced recommendations, reliable standards, and proper safety evaluation criteria. This work could provide a reference and direct the follow-up research, development, and application of microalgae (MA)-based processes for resource recovery from wastewater.

Keywords: microalgae culture technologies; wastewater treatment; resource recovery; research; development; application

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1. Introduction

In the circular economy, recovery and recycling of resources are becoming more critical perspectives in wastewater treatment beyond reducing the contaminants [1,2]. Carbon neutrality is presently a much-debated topic for wastewater treatment plants, involving conversations around the reduction of energy consumption, energy resources recovery, and greenhouse gas (GHG) emissions [3–5]. Under these global circumstances, technological innovations for resource recovery are essential in wastewater treatment. Over the past two decades, major drivers, such as the purpose of reducing operating costs in wastewater treatment and the gradual depletion of natural resources for fertilizer production, have emerged to improve resource recovery from wastewater [1,6]. The amount of nutrients available in wastewater is substantial [7,8]. Robles et al. (2020) estimate that the global recovery of phosphorus (P) and nitrogen (N) from waste streams could retrieve P consumed by humans and not dump it into the environment and could serve around 50% of the present

N market when assuming nitrogen to phosphorus mass ratio of 4:1 in the wastewater. Studies have confirmed that microalgae could uptake nutrients (e.g., N and P) and carbon (C) from wastewater [7].

Moreover, the application of wastewater as a nutrient source and CO_2 as a carbon source is becoming a low-cost strategy for scaled-up MA production [9]. MA cultures generate energy (i.e., ATP) from light and effectively assimilate C through photoautotrophic growth with UV-VIS 400–700 nm with the main feature of high biomass yields [10,11]. Indeed, in the last decade, there has been a noticeable scientific interest in microalgae-based technology for nutrient recovery from wastewaters [12], with a 20-fold increase in the number of publications on microalgae research worldwide [13]. When using MA as feedstock or others, the biomass production of MA from wastewater can bring a significant addition to value [13]. Hence, MA cultivation based on wastewater could provide considerable benefits over traditional wastewater treatment processes [14,15]: (1) The cost of wastewater treatment could be reduced; (2) MA cultivation could reduce the level of nutrients in wastewater to a low level to meet the stringent standards; (3) MA could live on nutrients provided in wastewater without or with limited supplementation; and (4) value-added products could be generated through MA cultivation, including feedstocks, biogas, fertilizers, and biofuels, sourced from CO_2, organic carbon sources, and nutrients from wastewater. Therefore, MA-based treatment processes have the potential to reduce the cost of wastewater treatment and fertilizer production while generating energy and reducing greenhouse gas (GHGs) emissions.

This paper reviews the research and developments of MA-based processes for treatment and resource recovery from various waste streams. On this basis, opinions on the MA species selection, the MA-based wastewater treatment system's configuration, influencing parameter adjusting, harvesting enhancement, and scale-up and promotion of MA-based processes for wastewater treatment are presented. It could serve as a reference and direct follow-up research, development, and application of MA-based wastewater treatment processes.

2. Mechanism, Culture, and Configuration in the Microalgal Wastewater Treatment Technologies

2.1. Enclosed Mechanisms of Microalgae for Nitrogen, Phosphorus, and Carbon Recovery

MA plays an important role in transforming inorganic N into an organic form by the process of assimilation. As illustrated in Figure 1, assimilation is a process performed by all eukaryotic algae that requires inorganic nitrogen to be in the form of nitrate, nitrite, and ammonium [16]. Inorganic nitrogen is first translocated, followed by the reduction of oxidized nitrogen and the incorporation of NH_4^+-N into amino acids. As has been confirmed, MA prefers NH_4^+-N over NO_3^--N, where NO_3^--N consumption would not occur until NH_4^+-N is nearly consumed completely [7,17]. The NH_4^+-N tolerance of different MA species varies in 25–1000 μmol N/L [18]. *P* is another important factor in the energy metabolism of MA, which is incorporated into ATP, phospholipids, and other organic compounds through multiple phosphorylations, accompanied with energy input [19]. During photosynthesis, autotrophic MA use CO_2 in the air as the carbon source and absorb N and P in wastewater (Equation (1)). Meanwhile, organic carbon sources could be metabolized without light in heterotrophic mode [9].

$$106CO_2 + 16NO_3{}^- + HPO_4{}^{2-} + 122H_2O + 18H^+ \overset{Sunlight}{\rightarrow} C_{106}H_{263}O_{110}N_{16}P + 138O_2 \quad (1)$$

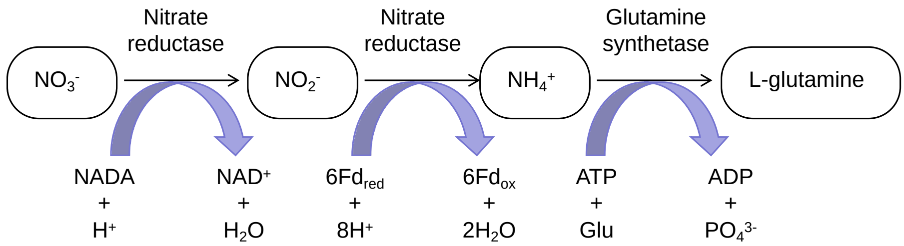

Figure 1. The process of N assimilation by MA.

Besides, in the coexisting system of bacteria and MA, mutual effect has been detected [20,21]. The organic pollutants in wastewater are decomposed by aerobic bacteria producing NH_4^+ and PO_4^{3-} and releasing CO_2, where MA can use NH_4^+, PO_4^{3-}, and CO_2 as nutrients releasing O_2 for bacteria [22–24] (Figure 2A). Therefore, this combination is recommended to enhance the removal of N and P, achieving both higher MA and bacteria biomass productions.

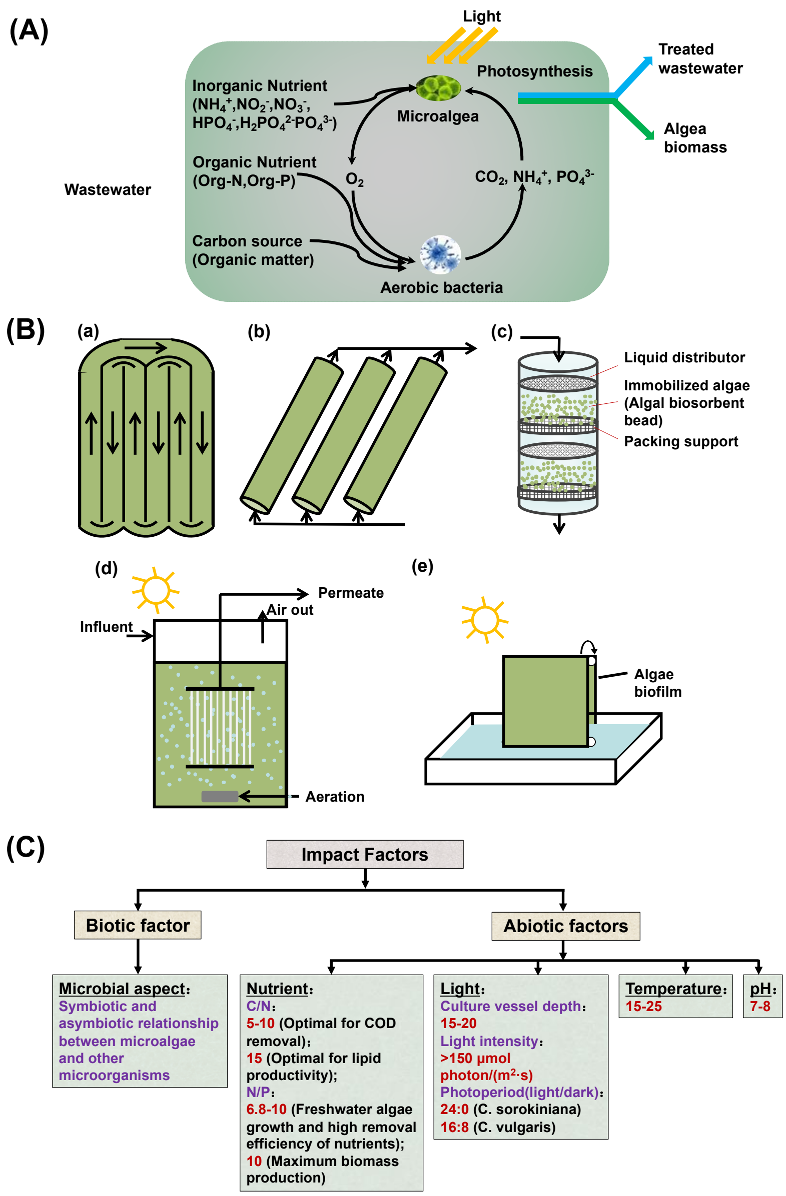

Figure 2. MA-based wastewater treatment: (**A**) Schematic nutrient uptake mechanism of the MA-bacteria system from wastewater; (**B**) Schematic diagrams of different photobioreactors ((**a**) suspended

open systems, (**b**) suspended closed systems, (**c**) immobilized algae system, (**d**) membrane photobioreactors, and (**e**) algae biofilm); (**C**) Factors affecting nutrients recovery from wastewater using MA.

2.2. Selection of Suitable Microalgae Cultures

Table 1 lists the properties of the MA species used for wastewater treatment [11,25–27]. According to the theoretic molecular formula of $C_{106}H_{263}O_{110}N_{16}P$ for MA, 0.063 g N and 0.009 g P are required for accumulating 1 g MA biomass, and C contributes over 50% the weight [11,22]. As Table 1 illustrates, the MA commonly used in wastewater treatment includes *Chlorella*, *Dunaliella*, *Scenedesmus*, *Spirulina*, etc. The biomass of all MA species mainly consists of carbohydrates, proteins, nucleic acids, and lipids, but their proportions varied a lot depending on the different MA types. It is worth noting that *Chlorella pyrenoidas*, *Chlorella vulgaris*, *Dunaliella salina*, *Dunaliella tertiolecta*, *Tricornutum*, *Scenedesmus obliquus*, *Spirulina platensis*, and *Spirulina maxima* have a protein composition of over 50% (dry *w*/*w*), which could have a higher N accumulating capability (Table 1); while *Chlorella protothecoides* showed the highest lipid productivity of 1214 mg/(L·d)), followed by the 116 mg/(L·d)) by *Dunaliella salina*. In terms of biomass productivity, *Chlorella protothecoides*, *Chlorella pyrenoidas*, and *Euglena gracilis* are leading among all the species with volumetric biomass productivities of 2000–7700, 2900–3640, and 7700 mg/(L·d), respectively (Table 1). Despite the composition and productivity, multiple criteria need to be considered in MA strain screening for wastewater treatment, which should involve high nutrient removal and biomass productivity, fast growth rate, strong adaptability to water characteristics, and local climate. When multiple criteria cannot be simultaneously met, "fast growth rate" should be the first choice, as it is usually positively related to high nutrient removal and high biomass and lipid productivity.

Table 1. The compositions and productivities of the commonly used MA species for wastewater treatment [11,25–27].

Representative MA Species	Composition (% dry *w*/*w*)			Lipid Productivity (mg/(L·d))	Biomass Productivity	
	Protein	Carbohydrate	Lipid (Oil)		Areal (g/(m·d))	Volumetric (mg/(L·d))
Botryococcus braunii	22	18	55–60	5.5	3	20
Chlorella protothecoides	10–20	12–20	55	1214	-	2000–7700
Chlorella pyrenoidas	54–60	24–28	11–12	-	72.5/130	2900–3640
Chlorella vulgaris	51–58	12–17	4–24/14–22	11.2–40	0.57–0.95	20–200
Dunaliella salina	57	32	6	116	1.6–3.5/20–38	220–340
Dunaliella tertiolecta	55–65	10–15	20	20	-	120
Euglena gracilis	39–61	14–18	14–20	-	-	7700
Phaedactulum Tricornutum	36.4–53.2	11.2–26.1	8–32.6	44.8	2.4–21	3–1900
Porphyridium cruentum	28–39	40–57	9–14	34.8	-	370
Scenedesmus obliquus	50–65	10–17/27	7/12–14	7.14/11.6–58.6	-	4–740
Scenedesmus quadricauda	4.4–9.5	3.7–24.8	6.9–10.6	35.1	-	190
Spirulina platensis	46–63	8–14	4 -9	-	1.5–18.0/24–51	60–430
Spirulina maxima	60–71	13–16	6–7	8.6	25	210–250

2.3. Configurations of the Microalgal Wastewater Treatment System

Design of photobioreactors is another key factor for obtaining both high efficiency and cost-effective MA-based wastewater treatment. As Figure 2B illustrated, the configurations are basically classified into a suspended open system (Figure 2B(a)), suspended closed system (Figure 2B(b)), immobilized algae system (Figure 2B(c)), and newly designed hybrid photobioreactors (Figure 2B(d)–(e)).

Currently, suspended open systems are the most commercial-scale algae cultivation system as they are easy to scale-up and have a relatively low capital cost (Figure 2B(a)) [28,29]. Some algae ponds are built on non-arable lands adjacent to power plants to get access to CO_2 from flue gas, and some are built near wastewater treatment plants to easily access nutrient supplies. However, the key issue of open ponds is the control process as it is easily affected by ambient factors and N/CO_2 losses, and a considerable area is required for construction [30]. Another issue for the open ponds is contamination, where the system needs to be started with large inoculum, and frequent harvesting and feeding is needed to maintain the dominance of functional MA strains. Moreover, sterilization is usually essential to reduce the negative effects of bacteria and pathogens on the growth of MA. As Figure 2B(b) shows, the development of suspended closed photobioreactors has helped to avoid evaporation and contamination, and increase photosynthesis efficiency when comparing open ponds [28]. The closed photobioreactors have been proven to be suitable for all mixotrophic, photoautotrophic, and heterotrophic MA cultures [31]. However, its high capital cost and energy consumption could only be afforded by the high value-added products.

Immobilization of algal cells in a solid medium (Figure 2B(c)) could prevent the MA mass from freely moving in the wastewater treatment system. It is a type of alternative method with the advantages of high removal rate and easier harvesting and water recycling. It should be noted that almost all studies on immobilization were carried out in laboratories since entrapment is the most frequently used method in such experiments which limits the knowledge of how such methods could work in larger contexts [7]. Compared with the free-living systems, the immobilized algal cells have higher uptake rates for both N and P [32]. Although several advantages have been demonstrated, cell behavior is most negatively affected by immobilization, particularly for the biomass growth rate and productivity [33]. Moreover, Ruiz-Marin et al. (2010) also proved that high immobilized cell density per bead and high immobilization matrix density could reduce the penetrating light and diminish metabolic activity.

The newly designed hybrid photobioreactors are focused on developing high efficiency systems to reduce capital and operational costs [34]. With this regard, the membrane photobioreactor (MPBR) has been proposed (Figure 2B(d)). MA cells, particularly Chlorella sp., are usually small in size and possess negligible relative density to water, making them hard to settle and form a homogeneous suspension [35]. In MPBR, the membrane could provide a complete MA cell retention, which could prevent cell washout and increase biomass concentration. Studies confirmed an effective retention of biomass by membrane thereby maintaining its growth in a total recycling system [36,37]. Another proposed newly designed hybrid system is MA biofilms (Figure 2B(e)). MA biofilms are consortiums of microorganisms that are wrapped in extracellular polymeric substances (EPS) [38]. Lee et al. (2014) proved that the system could achieve a biomass productivity that was 2.8-folds higher than that of the suspended system [39]. The enhanced biomass productivity could provide a much easier harvesting process and recovery procedure [7], which also indicated a significant reduction in operation costs [40].

3. Factors Influencing the Performance of Microalgal Wastewater System

3.1. Biotic Consortia and Infections

Biotic factors indicate the effect of other living microorganisms on the growth of MA or changing the wastewater treatment systems (Figure 2C). In wastewater treatment, microbial systems mainly consist of approximately 25 microorganisms communities, such as bacteria, fungus, algae, viruses, lichens, rotifers, and zooplanktons [41,42]. The symbiotic-asymbiotic relationships between MA and other microorganisms in wastewater could directly affect MA cultivation, nutrient recovery, and biomass production [42]. Hence, biotic consortia types dictate the relationship between MA and other microorganisms. Regarding wastewater treatment, bacteria are usually necessary and can be beneficial to microalgae cultivation [42]. Bacteria are capable of supporting the photoautotrophic growth of microalgae by providing CO_2 through their heterotrophic metabolism of organic matters, mineralizing them into inorganic compounds, e.g., NH_4^+ and PO_4^{3-}, which could be directly utilized by MA [41]. On the contrary, bacteria will compete with MA and have negative effects when nutrients are deficient.

Additionally, some MA species have antibacterial activities to the growth of bacteria. Meanwhile, a few bacterial antibacterial activities could inhibit MA growth. For instance, some cyanobacteria could produce inhibitory substances to the growth of eukaryotic algae, and some eukaryotic algae can produce antibacterial substances [43,44]. Similarly, some prokaryotes cause adverse conditions for algal growth [23]. Therefore, the type of biotic consortia that is present dictates the nature of the relationship between MA species and other microorganisms.

Infections are hard to avoid in open wastewater treatment systems, thus keeping optimal conditions for the algae are required for less susceptibility. Acidification of the cultures (to pH 2) for a short period or daily removal of particulate matter (larger than 100 µm) are proper methods used to avoid infections of zooplankton [45,46]. Acidification is adequate to kill most rotifers and protozoa, but it is difficult to realize in large ponds. Establishing a pond regime that could lead to diurnal anaerobic conditions for a short period could also prevent the development of animal and fungal populations, and a short period of high NH_4^+ concentration can eliminate contamination by zooplankton [43,47]. Moreover, biocides can be applied to avoid infection, but the cost and the potential spoiling of products should be considered [48].

3.2. Nutrient Balance

Nutrient balance, which mainly refers to the balance of C/N and N/P ratios, is critical for MA cultivation, biomass productivity, and the dominance of functional species in MA culture (Figure 2C). As Table 2 shows, C/N and N/P ratios varied greatly with the wastewater types. They are in the ranges of 0.67–2.16 and 5.68–19.3, respectively, in the municipal wastewater, but could be over six and below one, respectively, in the sewage concentrate and sludge centrifuge centrate. Animal wastewater usually has a higher total nitrogen (TN) concentration than municipal wastewater with a high N/P ratio, which could reach 48.72. Comparatively, industrial waste streams are more complicated than municipal wastewater and animal wastewater. For instance, in starch wastewater and digested starch wastewater, the C/N ratio can be as high as 858.82 and 175.09 with extremely low N/P ratios of 0.12 and 0.14, respectively (Table 2). Therefore, nutrient balancing is essential and a convenient way to promote nutrient assimilation and pollutant removal in the MA-based wastewater treatment system.

Researchers mixed the piggery wastewater with brewery wastewater with the purpose of achieving a balanced C/N ratio (7.9). The processes achieved 2.85 g/L biomass productivity with COD, TN, and total phosphorus (TP) removal of 93%, 96%, and 90%, respectively [49]. The study also showed that maximum MA production could be obtained at an N/P ratio of 10 [50]. However, Liu and Vyverman (2015) demonstrated that different strains showed different nutrient uptake capacities under varying N/P conditions [51]. Liu and Vyverman (2015) studied the differences in nutrient uptake capacity of the three different strains of benthic filamentous algae, i.e., *Cladophora*, *Klebsormidium*, and *Pseudanabaena*, under varying N:P conditions and observed a significant influence of the N:P ratio on algal growth and the phosphorous uptake process. The appropriate N:P ratios were 5:15, 7:10, and 7:20, for *Cladophora*, *Klebsormidium*, and *Pseudanabaena* strains, respectively [51]. The *Cladophora* strain exhibited the highest biomass production, while the *Pseudanabaena* strain had the largest N and P content. This study strongly suggested that the *Cladophora* strain had a great capacity to remove P from wastewater with a lower N:P ratio. Conversely, the *Pseudanabaena* strain was suitable for removing N from wastewater with a high N:P ratio. Therefore, a strain screen process is strongly suggested together with C/N and N/P balancing in the treatment of different wastewaters by the MA-based technologies.

Table 2. Different waste streams with various C/N and N/P ratios.

Wastewater Sources	Description	COD (mg/L)	TN (mg/L)	TP (mg/L)	C/N Ratio	N/P Ratio	Reference
Municipal wastewater	Sewage centrate	846 ± 12	48.6 ± 1.8	49.8 ± 2.2	6.53	0.98	[52]
	Fresh urine	-	5015 ± 209	347 ± 2	-	19.3	[53]
	Raw sewage	231.0 ± 4.2	40.65 ± 0.07	5.66 ± 0.08	2.13	7.18	[54]
	Primary settled sewage	224.0 ± 4.2	38.95 ± 1.91	6.86 ± 0.05	2.16	5.68	[54]
	Sludge centrifuge centrate	2250 ± 99	131.5 ± 2.1	201.5 ± 10.6	6.42	0.65	[54]
	Pretreated urban wastewater	150.0	84.42 ± 2.65	6.07 ± 0.26	0.67	13.91	[55]
	Disposing effluent	90	36.44 ± 1.93	2.38 ± 0.1	0.93	15.31	[55]
	Effluent from primary settler	160	33.9 ± 0.83	3.20 ± 0.1	1.77	10.59	[55]
Animal wastewater	Swine wastewater	12,000	1700	80	2.65	21.25	[56]
	Anaerobic digestate of swine manure	-	1218 NH_4^+-N	25	-	48.72	[57]
	Raw swine wastewater	1421	326.60 ± 2.98	74 ± 0.42	1.63	4.41	[58]
	Dairy manure	38,230	3305 TKN	266	4.34	12.42	[59]
	Digested dairy manure	23,760	3456 TKN	249.7	2.58	13.84	[59]
	Pretreated piggery	840 ± 15	512 ± 9	57 ± 1	0.62	8.98	[60]
	Digested piggery effluent	12,152	3304 TKN	192	1.38	17.21	[61]
	Digested pig waste	2746–4157	1405–1519	164–620	0.85–1.16	2.21–7.43	[62]
Industrial wastewater	Anaerobically-digested thin-stillage	4540	130.9 NH_4^+-N	21.5	13	6.09	[63]
	Brewery wastewater	547–6730	9–480	5–45	0.8–70.1	1.4–10.7	[49]
	Molasses wastewater	514,000	458	67	420.85	6.84	[64]
	Starch wastewater	5130 ± 1280	2.24 NH_4^+-N	18.3 ± 2.95	858.82	0.12	[65]
	Digested starch wastewater	1340 ± 520	2.87 NH_4^+-N	21.0 ± 4.21	175.09	0.14	[65]
	Soybean processing wastewater	8087–13,215	189.9–267.1	45.6–56.3	16.0–18.6	4.16–4.74	[10]
	Slaughterhouse wastewater	734–3560	64.8- 327.6	5.6–46.8	3.2–8.4	1.4–21.0	[66]

3.3. Operational Parameters

3.3.1. Light Intensity and Photoperiod

Light, temperature, and pH are the key operating parameters that affect the MA process (Figure 2C). Table 3 summaries relevant studies on the effect of light intensity as well as illumination cycles on algal growth and biomass productivity. The light intensity and photoperiod are the primary factors related to nutrient removal, MA growth rate, and biomass productivity [63]. Even the same strain with different light intensities or photoperiods displayed different performances. Additionally, different MA strains will have different capabilities to habituate under different irradiation levels [67].

As Table 3 shows, different *Chlorella* strains demonstrate a significant difference in biomass production when subjected to a diverse range of photoperiod regimes and various light intensities. Although some researchers found that enhanced light intensity can compromise the phosphorus removal capacity of MA, only acid-soluble polyphosphate is influenced by light intensity, indicating that strong light intensity would accelerate the consumption of acid-soluble polyphosphate [58]. Bazdar et al. (2018) found that *Chlorella. vulgaris* could obtain a high biomass productivity of 3.8 g/L at the light intensity of 7000 lx and light:dark ratio of 24:0 [68]. Researchers also investigated nutrient removal and biomass production using MA-based consortia under different photoperiod conditions, and the results revealed that carbon removal was positively related to the length of dark cycles while N and P presented the opposite trends, indicating the light-dark cycle as a key parameter for MA-based wastewater treatment [56,69].

Table 3. Studies on the effect of light intensities and photoperiods on the performance of MA-based wastewater treatment technologies.

Microalgae Species	Light Intensity (μmol/m^2/s)	Photoperiod (Light/Dark Ratio)	Specific Growth Rate (d^{-1})	Biomass Productivity	References
Chlorella. vulgaris	642	Natural light 15:9	-	14.05 g/m^2/day	[70]
	261	Natural light 15:9	-	8.09 g/m^2/day	
Chlorella. vulgaris	3500 lx	24:0	0.129	3.3 g/L	[68]
	5000 lx	24:0	0.136	3.6 g/L	
	7000 lx	24:0	0.143	3.8 g/L	
	5000 lx	16:8	-	3.2 g/L	
	5000 lx	12:12	-	2.7 g/L	
Chlorella. vulgaris	80	24:0		1.51 g/L	[2,56]
	110	24:0		1.79 g/L	
	140	24:0		1.87 g/L	
Chlorella. vulgaris	100	24:0	-	0.03 g/L/day	[71]
Chlorella. vulgaris	8000 lx	24:0	-	0.072 g/L/day	[72]
Scenedesmus. quadricauda	7000 lx	12:12	0.6	0.995 g/L	[73]
Spirulina platensis	3000 lx	24:0	0.16	1.70 g/L	[58]
Chlorella. sorokiniana	210	13:11	-	1.63 g/L	[63]
Chlorella sp.	370–430	12:12	0.19	6.8 g/m^2/day	[74]
		24:0	0.32	15.6 g/m^2/day	
Scenedesmus sp.	1300 lx	12:12	-	414.47 mg/L	[56]
Cyanobacteria, *Chlorella* sp. and *Scenedesmus* sp.	20	24:0	-	72% N recovery	[69]
	50	24:0	-	44% N recovery	
	100	24:0	-	46% N recovery	
Scenedesmus quadricauda	100	24:24	0.3	1.5 g/L	[75]
	500	24:24	1.057	4 g/L	
	1000	24:24	0.8	2 g/L	
	500	1:1	0.85	3.5 g/L	

3.3.2. Temperature

Temperature is also a crucial impact factor of MA-based wastewater treatment processes. Most MA can survive in the temperature range of 10–30 °C, usually with an optimal temperature range of 15–25 °C [76]. However, it varies according to the species. For example, thermophilic microalgae can survive at 35–40 °C. Researchers have grown *Chlorella minutissima* in the temperature range of 10–35 °C, finding that its growth rate increased up to 30 °C, but its specific growth rate started to decrease at 35 °C [77,78]. RuizMartínez et al. (2015) assessed the NH_4^+-N removal rate by *Scenedesmus* sp. at various temperatures from the effluent of a pilot-scale-submerged-anaerobic-membrane-bioreactor, finding that the NH_4^+-N remova increased with temperatures of 18 °C, 26 °C, and 34 °C, demonstrating rates of 6.7, 15.7, and 17.0 mg N/(L·d), respectively [79]. Filippino et al. (2015) reported a high efficiency in nutrient removal within a shorter cultivation period by *Chlorella. vulgaris* at a lower temperature, where an over 90% TN and TP reduction was achieved within four days of cultivation at 15 °C versus 12 days at 25 °C [80]. Therefore, the optimal temperature has been shown to vary depending on the microalgal species and their acclimation to a particular environment, and the main goal is to select the strain with more adaptive properties to a wide range of temperatures to achieve better cultivation.

Temperature control is challenging when using an open reactor cultivation system as seasonal variations cause relatively large fluctuations in temperature. Therefore, open systems are more favorable in areas where the temperature remains constant throughout the year [76,81]. MA in open reactors in a low temperature environment, especially in countries with a cold climate, are constrained as they are susceptible to photoinhibition at a winter light intensity of $\leq$600 μmol/m^2/s. Closed systems are recommended under this environment, where temperature could be controlled at the range of 20–30 °C, corresponding with the range of temperatures that promote growth rate and nutrient removal, making them an ideal choice for low temperature regions [77,82].

3.3.3. pH

pH showed a marked impact on MA-based wastewater treatment processes [9,83]. The MA-based systems usually contain MA, bacteria, pathogens, and many other microorganisms, all of which are sensitive to changes in pH [83]. The preferred pH range for MA is 7–8, yet some species can adapt to a wider pH range of 4–11; for instance, *Spirulina* could survive at a pH range up to 11–12 [83]. As Han et al. (2013) demonstrated, *Chlorella pyrenoidosa* could deliver the best results of biomass and lipids production at a pH of seven under 30 °C [84]. Algae intracellular pH is neutral and a proton exchange phenomenon regulates its internal cytoplasmic pH [9]. Thus, algae might protect itself from pH damage with mucous coating and by excreting acidic waste to neutralize the underlying conditions to preserve its microenvironment. Even if the pH of the media used is alkaline, the cytoplasmic pH of MA stays near neutral, which is achieved by homeostasis involving proton efflux/influx mechanisms. However, external pH adjusting is essential when the pH of wastewater is out of the self-regulation range of MA [83].

4. Pathways to Enhance Microalgae Biomass Harvesting

Numerous methods of harvesting have been investigated for MA processes, including adherence techniques, e.g., coagulation, flocculation, and flotation, and force applications, e.g., centrifugation and filtration [85]. Flocculation has been considered a promising method which can harvest high amounts of MA biomass. Inorganic flocculants (e.g., aluminum sulfate, aluminum polychloride, and ferric chloride) are utilized as coagulants, but these flocculants usually result in biomass contamination and generate toxic and nonbiodegradable downstream products, causing health implications to the aquatic system and human life [86,87]. The development of new flocculants based on natural biodegradable raw materials is important for designing efficient and low toxicity agents to replace chemicals. Organic flocculants extracted from plants e.g., Moringa oleifera [88], Margaritarea discoidea [89], Cactus latifaria [90], chitosan [91], and vegetable tannins [92] have been studied and have shown efficiency as flocculant agents for water treatment or for MA biomass harvesting.

Moringa oleifera seeds have been recognized as one of the best natural flocculants for the treatment of turbid waters. Its high flocculating power is attributed to the cationic protein contained in the seeds as an active biocoagulating component for the treatment of wastewater, which destabilizes the particles contained in the water and flocculates the colloids through a process of neutralization and adsorption followed by sedimentation [87,93]. Moraes et al. (2021) showed the potential of Moringa oleifera seed flocculants for *Chlorella vulgaris*. This work demonstrated substantial added value in MA lipid content with the use of Moringa oleifera seeds as a flocculant agent besides the high efficiency of biomass recovery. The analyses of the biomasses produced in residual media demonstrated the possibility of recycling them, which is an important step to reduce the water footprint and process cost of microalgae biomass production [87].

Chitosan is also a potential biocoagulant to replace chemical coagulants with high flocculation ability [94]. It is known to have positive charges and can be used to coagulate a microalgae cell that has a negative charge. It is also biodegradable, and Mohd Yunos et al. (2017b) proved that a low dosage and short settling time are required in MA harvesting [91]. Despite the use of bioproducts, Nguyen et al. (2019) used the bacteria in seafood wastewater treatment effluent directly for bioflocculation to harvest *C. vulgaris*. The direct use of untreated seafood wastewater treatment effluent as a culture medium for *C. vulgaris* allowed a flocculation activity of 92.0 $\pm$ 6.0% and nutrient removal of 88.0 $\pm$ 2.2%. The microalgal-bacterial flocs collected under this optimal condition contained dry matter of 107.2 $\pm$ 5.6 g/L and chlorophyll content of 25.5 $\pm$ 0.2 mg/L [95]. The direct use of bacteria in the treated effluent has been more convenient and cost-effective than using bioproducts.

The application of natural flocculants could solve the major problems of biomass contamination and medium reuse, reducing environmental impact and water footprint during MA production. Besides speeding up MA harvesting, the microalgal-bacterial flocs could adsorb suspended compounds in the surrounding medium to form co-bioflocculate and enhance the removal of nutrients [87,96]. Although various advantages have been presented, the selection of biocoagulants and operating parameter optimization depending on the treatment conditions are critical to ensure an efficient and cost-effective application.

5. Application of Microalgal Processes for Various Wastewater Treatment

Table 4 summarized the applications and performances of a variety of MA-based systems from 2010 to 2022. As Table 4 shows, MA technology has been applied to treat various waste streams, including municipal wastewater, dairy wastewater, swine wastewater, anaerobic digestate, piggery wastewater, winery wastewater, urine, soybean processing wastewater, and so on. It is a promising alternative for nutrient assimilation and recovery from wastewater, with high-value biomass production, competitive yields, and a small carbon footprint (Table 4). However, MA-based technologies are mainly in laboratory experiments at reaction volumes of 0.1–5 L and upscaled practical applications have rarely been found.

Challenges remain in upscaling for maintaining their cost-effectiveness and high efficiency [97]. Romero-Villegas et al. (2018) set up a high-rate algal pond to treat the diluted centrate from sewage. The system has a reaction volume of 855 L and obtained TN and TP removal 90% and 82% at the initial NH_4^+-N and TP concentrations of 700 mg/L and 11.5 mg/L, respectively [98]. However, the cost and environmental life cycle assessments on the processes have not been found. It still lacks enough environmental and economic feasibility assessments for photosynthetic-based systems in the cases of different wastewater sources and implementation scenarios. Further improvement in the availability of technologies is required by reducing the occupation area and operational cost, thereby maximizing light availability and biomass separation.

As Table 4 illustrated, the MA technologies varied a lot in different studies. Even in the treatment of the same or similar types of wastewaters, different reactor types, MA species, and operational conditions have been applied, thus obtaining varied performances. Therefore, more comparisons across different MA technologies on the treatment of a certain type of wastewater are required with the evaluation of efficiency, cost, and safety. There is potential to further develop systematic recommendations or application standards, which would significantly benefit the scale-up, promotion, and implementation of MA-based systems for the treatment of various types of waste streams.

Table 4. The applications and performances of a variety of microalgae-based systems within the years 2010–2022.

Wastewater Type	Reactor/Operation Type	Working Volume (L)	Microalgae Species	Operating Conditions	Treatment Time (day)	Initial TN (mg/L)	TN Removal (%)	Initial TP (mg/L)	TP Removal (%)	Reference
Municipal wastewater	PBR; batch	0.3	*Chlorella. vulgaris*	300 rpm; IR: 2000 lux	14	40 NH_4^+-N	100	10	45	[99]
Municipal sewage	Batch	-	*Chlorella* & *Scenedesmus*	30 ± 1 °C; IR: all-day light 4000 lx or dark	7	49.4 NH_4^+-N	97 NH_4^+-N	9.5	100	[100]
Municipal wastewater	Sequencing MPBR	5	*Euglena* sp.	SRT: 60 days; HRT: 2–8 days	2–8	24.7 ± 0.5	82.8–96%	3.5±0.5	35.7–70%	[101]
Municipal wastewater	MPBR	4	*Chlorella. vulgaris*	25–30 °C; IR: 120.8 μmole·m^{-2}·s^{-1}; pH: 6.5–7.8	35	14.12 ± 0.95	87.7	0.78 ± 0.11	76.7	[102]
Dairy wastewater	Batch	3	*Chlorella* sp. *Chlorella. sorokiniana*	30 °C; aeration at 1 vvm; 150 rpm; IR: 250 μE·m^{-2}·s^{-1}; Light/darkratio = 16:8,	10	1750 NO_3^--N	85	55	100	[103]
Digested dairy manure wastewater	Flask; batch	0.1	*Chlorella* sp.	25 ± 2 °C; 150 rpm; continuous fluorescent light illumination	21	109–239	75.7–82.5	15.3–29.5	62.5–74.7	[59]
Swine wastewater	Flask; batch	-	*Spirulina platensis*	IR: all day light 3000 ± 100 lux; pH: 8.45 ± 0.01	15	326.60 ± 2.98	91.24	74 ± 0.42	87.44	[58]
Swine wastewater	Beaker; batch	0.15	*Scenedesmus* sp.	25 °C; IR: 1300 lx; 12 h light/12 h dark	10	1700	60.75	80	96.13	[56]
Anaerobic digestate of swine manure	Column PBR	1.5	*Chlorella. vulgaris*	25 °C; IR: 140 μmole·m^{-2}·s^{-1},	7	1218 NH_4^+-N	95.12 NH_4^+-N	25	76.87	[57]
Piggery wastewater	Flask; batch	0.8	*Desmodesmus* sp.	25 °C; 150 rpm mixing; IR: 8000 lx	8	393.82 ± 15.98	52	15.61 ± 0.76	100	[104]
Mixed piggery-brewery wastewater	Flask; batch	0.75	*Chlorella. vulgaris*	25 °C; IR: 200 μmole·m^{-2}·s^{-1}; 12 h light/12 h dark	7	9–480	32–96	5–45	28–95	[49]
Winery wastewater	Hybridization tubes; batch	0.2	a. *Chlorella. Sorokiniana* b. *Auxenochlorella protothecoides*	28 °C; 150 rpm mixing; pH: 7.5; mixed CO_2 125 mL/min	5	a. 114 NH_4^+-N b. 114 NH_4^+-N	a. 100 100	a. 44 44	a. 100 100	[105]
Anaerobically-digested thin-stillage	Glass bottle; batch	1	*Chlorella. sorokiniana*	23 ± 2 °C; 400 rpm; IR: 210 μmole·m^{-2}·s^{-1}; 13 h light/11 h dark; Mixed 2% CO_2: 0.01 vvm	18	130.9 NH_4^+-N	95.3 NH_4^+-N	21.5	78.3	[63]

Table 4. *Cont.*

Wastewater Type	Reactor/Operation Type	Working Volume (L)	Microalgae Species	Operating Conditions	Treatment Time (day)	Initial TN (mg/L)	TN Removal (%)	Initial TP (mg/L)	TP Removal (%)	Reference
Fresh urine	PBR;	4	*Chlorella. vulgaris*	IR: 3000 lx; 24 h light/24 h dark; CO_2/air mixture at flow rate of 2 L/min	7	50.5	77.3	4.7	53.2	[53]
Soybean processing wastewater	Flask; batch and fed-batch	0.1	*Chlorella. pyrenoidosa*	25 ± 1 °C; intermittent shaking IR: 27 μmole $m^{-2}·s^{-1}$; light/dark ratio = 14:10	5	16.8–17.5	88.8	16.8–17.5	70.3	[10]
Diluted centrate from sewage	High-rate algal pond; semi-continuous	855	*Nannochloropsis gaditana*	39 °C; pH: 7.3–8.2; air flow rate: 0.3 $v·v^{-1}·min^{-1}$; IR: 20–88 $μE·m^{-2}·s^{-1}$	1/6	700 NH_4^+-N	90	11.5	82	[98]
Agricultural wastewater	MPBR	4	*Chlorella. Vulgaris*	25 ± 2 °C; IR: 120.8 $μmole·m^{-2}·s^{-1}$; pH: 6.8–7.2	16	6.81 ± 0.68	86.1%	0.42 ± 0.05	82.7%	[106]

6. Perspectives

There have been many studies conducted on the application and feasibility of MA-based wastewater treatment processes. However, most of these studies have still been conducted on a laboratory scale without considering the feasibility of scaling up to pilot or larger scales. Scaling up is strongly connected with cost accounting, including investment and operational costs, and the performance of the process may significantly varied differences when scaled up. One major challenge for large-scale wastewater treatment plants is the inhibition of microalgal growth due to dark-colored wastewater and hazardous pollutants. To break this limitation, it is recommended to control influents through proper input or pretreatment before introducing microalgae. Furthermore, wastewater contains a vast diversity of nutrients with varying strengths, requiring multiple pretreatments to achieve an ideal nutrient balance for microalgae. Another limitation of MA-based processes is the lack of economic and environmental feasibility assessments. Very few studies have analyzed and discussed the costs and benefits of MA-based wastewater treatment processes, making environmental life cycle analyses necessary for various wastewater scenarios. To enhance the economic feasibility of MA-based processes, labor cost reduction and more automated designs should be considered. Efforts are needed to promote the upscaling and successful implementation of MA-based wastewater treatment processes. One way to achieve this is to establish basic rules, advanced recommendations, and reliability standards that enhance the adaptability of these processes. It is also essential to develop proper safety evaluation criteria and utilization guidelines for cultured microalgae. By implementing these measures, the widespread adoption of MA-based wastewater treatment processes can be achieved, ensuring their safety and efficacy.

7. Conclusions

The MA-based process is a promising alternative for nutrient recovery from wastewater, and it possesses the potential for cost reduction of wastewater treatment and fertilizer production, energy generation, and GHGs emission reduction. In MA species screening, "fast growth rate" should be the first choice. In terms of configuration, systems that enable easier MA separation, harvesting, and dewatering should be developed, which have the potential to reduce operational costs. The types of biotic consortia and infections need to be considered and controlled depending on the MA species and ambient conditions. Nutrient balance adjusting and strain screen processes for different types of waste streams with various C, N, and P conditions are required. The optimization of light intensity, photoperiod regimes, temperature, and pH is essential to obtain an efficient MA system. In terms of the enhancement of MA biomass harvesting, the application of bioflocculant has shown considerable potential, but the biocoagulants' selection and harvesting parameter optimization is critical to ensure an efficient and cost-effective application. In addition, the successful implementation of the MA-based wastewater treatment process also depends on the design and optimization of the process, taking into account the characteristics of the wastewater and the specific preferences of the microalgae species being used. Minimizing costs and achieving economic feasibility are crucial factors that must be considered to ensure the successful implementation of MA-based wastewater treatment processes.

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