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REVIEW

Suspended filamentous algal cultures for wastewater treatment: A review

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Abstract
More than 50 years have passed since the first studies of microalgae treating effluents were published. Suspended growth of filamentous algae in wastewater ponds has been considered in several publications for over a decade. However, despite all the research efforts and the knowledge generated, the technology is far from being adopted. This review compiles all the publications identified in different databases, which used filamentous algal ponds to remediate varied wastewaters, with the aim of identifying the research needs to allow the technology's application. The experimental methods and results obtained were extracted and compiled for comparison from 28 relevant studies, in which municipal wastewater and *Oedogonium* spp. were most used. Most of the studies were performed at a small laboratory scale and for short time periods. There was a remarkable use of effluents with a high degree of pretreatment and more studies focused on the biomass productivity than the treatment performance. It is recommended that future research use wastewater, with minimum intervention, rather than defined nutrient solutions, to assess the potential for wastewater treatment. Transitioning from laboratory to outdoor systems at scale should be a primary aim to further adopt this technology.

Keywords Effluent · Filamentous algal ponds · Phycoremediation · Wastewater treatment · Bioremediation

Introduction

Wastewater treatment using algal cultures is a field that has many decades of history, yet it still must overcome many bottlenecks to reach an extended application. High-rate algal ponds are a proven, effective microalgal treatment technology (Young et al. 2017; Fallowfield et al. 2018), however, there is still potential for improvement. The application of microalgae for wastewater treatment predominates in data in current studies, which to meet discharge standards requires biomass removal, contributing to a high percentage of both the economic and energetic final cost of the process (Acien et al. 2016). Therefore, cost-effective removal methods are needed to enhance this technology's application. There are studies, which evaluate alternative methods for the separation of microalgal biomass from the treated wastewater (Park et al. 2019; Chu et al. 2021; Young et al. 2021). In contrast, there has been less focus on application of filamentous algae to merge wastewater treatment with biomass production (Lawton et al. 2017). Compared to microalgae, filamentous algae offer the advantage of facilitating biomass separation that would reduce the cost and complexity of the technologies employed, lowering both the capital and operational expenditures (CAPEX and OPEX respectively) of potential projects employing this technology. However, there are still many research needs to address before adoption of freshwater filamentous algae for wastewater treatment (Liu et al. 2020; Rearte et al. 2021), especially regarding large scale outdoor validation, the effect of nutrient loading, operational parameters and other aspects that will be reviewed here.

There are already some successful systems utilising filamentous algae, consisting of inclined planes where a matrix supports attached growth, often known as algal turf scrubbers (ATS) (Mulbry et al. 2010), which are already commercialized. Numerous studies have been conducted with these types of devices, which have also identified specific challenges to that approach (Blersch et al. 2013). This

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review, however, will not include ATS given that it is considered these are designed to treat specific wastewater types (WW). Furthermore, the short hydraulic retention time of ATS would be insufficient for many filamentous algae to effectively remove nutrients or contaminants from more concentrated effluents.

This review provides a state-of-the-art overview of wastewater treatment using filamentous algae growing suspended in various wastewater matrices. The studies and findings were compared and discussed when considering: the type of wastewater used and the degree of pretreatment received, the filamentous algae employed and their performance, and the environmental conditions in which those studies were conducted. The review also identifies the research needed to further the application and adoption of filamentous algae for wastewater treatment.

Methods

A literature review was conducted using the keywords “filamentous algae”, “macroalgae”, “wastewater”, “wastewater treatment” and “HRAP” in Scopus, ScienceDirect, Taylor & Francis Online, SpringerLINK and Wiley Online Library. The criteria to incorporate publications into this review were as follows: (1) the studies must have been conducted with filamentous algae suspended in the matrix, (2) the studies must have assessed filamentous algae growth or nutrient removal in the matrix, (3) at least one of the goals must have been to build knowledge towards wastewater treatment (WWT) systems. An exemption was made to include the study performed by Kim et al., (2018) because, despite the filamentous algae growing attached to the sand bottom, the biomass was spread across the water column and the depth and operational parameters of the pond were more similar to HRAPs than biofilm filters or ATSs. The references cited in the articles retrieved on the first search were also interrogated for other relevant articles. The data were collated to enable detailed review of the ‘state of the art’ of the field and identify future research needs. Note that the data was organised by case study, hence there were publications with more than one reported study.

Wastewater types and their manipulation

A wide spectrum of effluents was utilised in studies reporting the performance of filamentous algae for WWT. This range could be partitioned in to three groups by their contaminant type. The first one being wastewater with high organic load, where nitrogen, phosphorus and carbon can be a problem if released in the environment without adequate treatment (e.g., triggering eutrophication processes). The second group could be those containing metals or metalloids. Heavy metals are toxic even at very low concentrations, which are often surpassed by industrial effluents. Finally, the organic pollutants group, which are basically organic substances toxic or harmful to humans, flora and fauna.

The most represented WW in this review belong to the first group - high organic load, where 19 out of 28 studies were carried out using effluents with the goal of lowering the N, P and C concentrations. Municipal WW was the most common effluent in this group (13 experiments were conducted with WW from this origin) (Yun et al. 2014; Cole et al. 2016; Neveux et al. 2016; Ge et al. 2018; Min et al. 2019; Piotrowski et al. 2020; Lawton et al. 2021; Kube et al. 2022), combined with a high variety of pre-treatment processes. Some effluents went through a settling and screening process only (Neveux et al. 2016), in most studies the wastewater was collected from a later treatment stage at the WWTP (Yun et al. 2014; Cole et al. 2016; Neveux et al. 2016; Ge et al. 2018; Lawton et al. 2021; Kube et al. 2022), some studies used the final effluent of the WWTP and performed further conditioning in the laboratory such as UV sterilization or autoclaving (Ge et al. 2018; Min et al. 2019; Piotrowski et al. 2020). The remainder of the studies used effluents from different industries or activities such as piggeries, slaughterhouses, rural runoff, and aquaculture (Saunders et al. 2012; Roberts et al. 2013, 2015, 2018; Wang et al. 2013; Cole et al. 2014, 2015; Ellison et al. 2014; Kim et al. 2018; Tan et al. 2018; Tabinda et al. 2019; Valero-Rodriguez et al. 2020; Rearte et al 2021; Ali Kubar et al. 2022; Liu et al. 2023) Table 1 and 2.

It is worth noting that two experiments (Valero-Rodriguez et al. 2020; Liu et al. 2023), purporting to explore distinct aspects of WW treatment/nutrient recycling used synthetic growth media. Even if the intention were to simulate the N, P, C load of an effluent, this is a questionable approach, given the heterogeneity which characterizes most wastewaters.

Conventional wastewater treatment plants (WWTP) have various stages of treatment where the effluents get progressively treated until the desired standards of quality are met. One of the aims of utilising filamentous algae is to replace as many steps as possible of the conventional treatment without sacrificing the final quality of the treated wastewater. The effluents used in the studies reviewed had a variety of pretreatment processes, however, some lacked detail regarding treatment and effluent composition, for example merely noting that “secondary treated effluent” was used in the study. The detailed reporting of any pre-treatment and subsequent composition of influents used in studies investigating the performance of algae for wastewater treatment should be encouraged.

Wastewater treatment processes may be designed for a myriad of situations, each with specific conditions and operational requirements. This approach could explain the

Table 1 Characterization and manipulation of wastewaters included in studies investigating the application of filamentous algae to wastewater treatment, where C, carbon; DIN, dissolved inorganic nitrogen; DRP, dissolved reactive phosphorus; TDN, total dissolved nitrogen; TOC, total organic carbon and NH_4-N, nitrogen as ammonium, nr, not reported

Wastewater	Previous treatment	CO_2 injection	Nutrient amendment	TN mg L^{-1}	TP mg L^{-1}	COD mg L^{-1}	Reference
ABS industry effluent	Filtrated, centrifuged, and 0.45 µm filtered	Yes		40	12	288	Ali Kubar et al. (2022)
Aquaculture effluent	Reed beds, settled and sand filtered to 150 µm	Yes	Yes	2.34	0.35	nr	Cole et al. (2014)
Aquaculture effluent	Reed beds, settled and sand filtered to 150 µm			2.62	0.56	nr	Cole et al. (2015)
Ash water	-	Yes	Yes	nr	nr	nr	Roberts et al. (2013)
Ash water	10 µm filtered	Yes, flue gases	Yes	nr	nr	nr	Roberts et al. (2015)
Ash water	-		Yes	nr	nr	nr	Saunders et al. (2012)
Ash water	-		Yes	nr	nr	nr	Ellison et al. (2014)
Contaminated rural streams	-		Yes	2.85	0.31	2.7	Kim et al. (2018)
Municipal	Fully treated, 10 µm filtered and UV sterilized	Yes		95.65	nr	nr	Piotrowski et al. (2020)
Municipal	5-stage Barden Pho process			5.3 DIN	2.6 DRP	nr	Lawton et al. (2021)
Municipal	Fully treated			1.12	0.23	12	Neveux et al. (2016)
Municipal	Fully treated			3.18	0.92	nr	Cole et al. (2016)
Municipal	Fully treated and autoclaved			9.9	0.6	13.4	Min et al. (2019)
Municipal	Aeration, filtering, UV disinfection, wetland			19.5 DIN	4.3 DRP	nr	Lawton et al. (2021)
Municipal	Primary and secondary treatment		Yes	23.75 TDN	6.77	27.5 dissolved C	Kube et al. (2022)
Municipal	Primary and secondary treatment			20.4	3.5	nr	Yun et al. (2014)
Municipal	Primary, Secondary, Autoclaved	Yes		10.7	1.47	14	Ge et al. (2018)
Municipal	Primary, Secondary, Autoclaved			10.7	1.47	14	Ge et al. (2018)
Municipal	Primary, Secondary, Diluted centrate, Autoclaved			16.06	0.63	10.26	Ge et al. (2018)
Municipal	Screened and settled			27.2	5.04	31	Neveux et al. (2016)
Municipal	Screened and settled			41.2	6.7	53	Neveux et al. (2016)
Piggery effluent	Digested and diluted			65.5	6.2	90.7	Wang et al (2013)
Piggery effluent	Settled, filtered and settled			486	149	1,474	Tan et al. (2018)
Slaughterhouse effluent	Settled and screened	Yes		61 NH_4-N	5.9 DRP	291 TOC	Rearte et al. (2021)
Synthetic growth medium	-		-	-	-	-	Valero-Rodriguez et al. (2020)
Synthetic growth medium	-		-	-	-	-	Liu et al. (2023)
Textile industry effluent	Unknown, diluted		Yes	nr	nr	1,140	Tabinda et al. (2019)
Water treatment residuals	Settled	Yes	Yes	nr	nr	nr	Roberts et al. (2018)

range of pre-treatments used since, based on the specific situation, filamentous algal treatment could be employed at different stages within the treatment train. Of the 26 studies conducted with 'real' WW, seven reported working with effluents, which had received three steps of conventional treatment, and six studies performed a sterilization in the laboratory (UV disinfection or autoclaving). Sterilisation will obviate any interaction between the metabolic activity of the algae and the effluent's microbial load. Furthermore, most of the pathogens would have been filtered out or eliminated in the sterilization stages before the algae could perform any treatment (Cole et al. 2016; Neveux et al. 2016; Ge et al. 2018; Min et al. 2019; Piotrowski et al. 2020; Lawton et al. 2021). Sterilization in the laboratory and other experimental procedures e.g., 0.45 µm filtration (Ali Kubar et al. 2022) are impracticable at scale and unlikely to be adopted at wastewater treatment plants. Such interventions highlight, when determining a study design, the importance of an awareness of the potential operating conditions under which the potential filamentous algae are required to perform.

To be able to compare the performance of different WW systems it is necessary to know the initial composition of the wastewater. Surprisingly one reviewed study carried out with 'real' wastewater did not report the initial values of C-N-P (Piotrowski et al. 2020). The concentrations of nitrogen, phosphorous and COD vary widely between influents used to investigate the application of filamentous algae to wastewater treatment. The reported values of different species of nitrogen and phosphorus from thirteen studies using 'real' municipal WW (Yun et al. 2014; Cole et al. 2016; Neveux et al. 2016; Ge et al. 2018; Min et al. 2019; Piotrowski et al. 2020; Lawton et al. 2021; Kube et al. 2022)) were transformed into concentrations of TN and TP and are, together with COD, shown in Fig. 1a. As can be seen, the TN ranges from a maximum of 41.2 mg TN L^{-1} for an effluent that only had screening and settling stages as pre-treatment (Neveux et al. 2016), to 1.12 mg TN L^{-1} for an effluent reported to be "fully treated" (Neveux et al. 2016). The maximum and minimum TP concentration reported were measured in the same WWs respectively, at 6.7 mg L^{-1} and 0.23 mg L^{-1}. Regarding the COD, the effluent used by (Neveux et al. 2016) presented the highest value at 53 mg COD L^{-1}, while the lowest value was reported by (Ge et al. 2018) at 10.26 mg COD L^{-1}, for municipal WW with primary and secondary pre-treatment stages followed by dilution and autoclave sterilization.

Across various studies reviewed there was a large variation of concentrations of TN, TP, and COD between effluents from different industries (Fig. 1b). Tan et al. (2018) reported the highest values of the measured parameters in piggery wastewater; 486 mg TN L^{-1}, 149 mg TP L^{-1} and 1474 mg COD L^{-1}. In contrast, Cole et al. (2015) reported the lowest initial values of nitrogen for aquaculture effluent; at 2.62 mg TN L^{-1} and Kim et al. (2018) reported the lowest phosphorus concentration in rural stream water; at 0.56 mg TP L^{-1}. However, there is still disparity across studies that clearly demonstrates the need to report the composition of the effluent used in the respective studies to enable adequate comparison of treatment performance (Fig. 1).

Almost a third of the experiments reported supplementing the wastewater with CO_2 through a sparging system (Roberts et al. 2013, 2015, 2018; Cole et al. 2014; Ge et al. 2018; Piotrowski et al. 2020; Rearte et al. 2021; Ali Kubar et al. 2022), in some cases the gas addition was at fixed rates and in others it was managed through pH control (Fig. 2). Employed as a strategy even in full scale HRAPs (Acién et al. 2016), the injection of CO_2 as an inorganic carbon source would be acceptable when the availability of assimilable inorganic carbon is limiting for photosynthesis (i.e., as in the case of some synthetic media). However, effluents with high organic load already have a generous concentration of organic carbon, which via microbial respiration can be converted to inorganic carbon available for photosynthesis. Further, this process contributes to one of the desired effects of the treatment process by lowering the organic carbon concentration, supplementing CO_2 could partially inhibit the aerobic metabolization of the organic carbon of the effluent, which is in the core of the biological treatment of WW.

More consideration should be given to the feasibility of upscaling experimental processes incorporating CO_2 addition to operational wastewater treatment systems. Specifically, the source of the gas supply and the infrastructure necessary to supplement the wastewater. Both of thse also have implications for increases in CAPEX and OPEX of the potential projects.

Flue gases may be a source of CO_2 for injection, including power stations using fossil fuels, noting that the outcome would contribute to carbon sequestration into biomass, reducing greenhouse gases emissions. This would require the wastewater treatment plant and the source of CO_2 to be in proximity, which may be a significant constraint. Furthermore, establishing a wastewater treatment system, which requires access to CO_2 generated from combustion of fossil fuels should not be considered a sustainable option. Furthermore, it is currently inconclusive whether the use of flue gases in algal cultures enhances biomass productivity and nutrient removal. Roberts et al. (2015) reported the outcome of using the flue gases from a coal-fired power station. Their results indicate that the C capture was negligible relative to the power station emissions and the growth rates achieved by the filamentous algae in the outdoor system were similar to cultures without CO_2 injection at smaller scales. These conclusions are similar to those of Young et al. (2019) using the CO_2 recovered from biogas scrubbing in pilot scale, outdoor microalgal HRAPs. Neither the wastewater treatment performance nor biomass production was significantly enhanced by the gas injection. The perspective of designing alternative, sustainable WWT systems that have lower environmental impact and rely on biological processes powered by solar energy is less attractive if they require added consumables.

Additional nutrients either as separate salts or growth media mixes were added to 25 % of the experimental systems in studies in this review. In this subgroup there were four studies treating ash water (Saunders et al. 2012; Roberts et al. 2013, 2015; Ellison et al. 2014), one using water treatment residuals containing high concentrations of aluminium (Roberts et al. 2018) and one remediating textile industry effluent (Tabinda et al. 2019). These effluents were amended because they did not contain enough C, N and P to support the algal growth. It is worth noting that the rationale in these cases was to supplement the WW with nutrients to enable filamentous algae to grow and sequester the metals intracellularly, followed by algal harvesting and extraction with the biomass.

Similarly, Kube et al. (2022) used municipal WW that had received primary and secondary treatment in a conventional WWTP and rather than using an effluent with high nutrient content, they subsequently added nutrients to maintain initial concentrations of 21 mg TN L^{-1} and 5 mg L^{-1} of phosphate. Kim et al. (2018) also amended the inlet water with nutrients intending to treat contaminated water from rural streams. The practical utility of amending wastewater with nutrients to enable algal growth is unclear. The practice increases the concentration of what are normally target contaminants for removal, requires a source of nutrients that have competing value for food production and poses the question whether the use of algae is an appropriate treatment option.

An additional aspect of the pretreatment to consider is the dilution of effluents. This strategy was employed in three studies (Wang et al. 2013; Ge et al. 2018; Tabinda et al. 2019) to lower the potential toxicity of certain contaminants, especially ammonia, with freshwater. This practice raises two issues, firstly, the potential WWTP would require the use of increasingly scarce, good quality water to treat contaminated effluents. Alternatively, effluents with lower concentrations of N-P could be considered for the dilution. Dilution also increases the volume of wastewater requiring treatment increasing the size of the facility required for treatment using filamentous algae. Ge et al. (2018) conducted experiments with 50 times diluted centrate from municipal WW and 5-fold diluted secondary treated municipal WW. Tabinda et al. (2019) performed studies on textile industry WW, which had to be diluted 7 to 20-fold. The digested piggery effluent used by Wang et al. (2013) was diluted 5 to 25-fold. The volume of the treatment pond would increase equivalent to the dilution factor compared with the undiluted effluent for the same hydraulic retention time.

However, the dilution of effluents may be justifiable at the beginning of a study, as in the case of batch cultures used to explore the dynamics of a potential continuous system. The rationale being that once a pond is stable at the applied hydraulic retention time and the exchanged volume is relatively small, dilution of the wastewater at the inlet will occur given that nutrient concentrations in the pond are likely reduced by algal growth. Another situation in which dilution of the wastewater is necessary is when determining the algal tolerance thresholds to nutrients or chemical concentrations. In order to assess the feasibility of algal treatment for some types of wastewaters it is necessary to know the upper and lower tolerance limits to the nutrients and contaminants within the influent. In any case, diluting the wastewater should be a strategy only applied at the early exploratory stages of the design of an algal based treatment process, and avoided in full scale WWTP operation.

Wastewater Treatment by Filamentous Algae

Chlorophyceae was the most common class of filamentous algae used for wastewater treatment (WWT) systems, with a single genus *Oedogonium* being used in 75 % of the studies (Fig. 3). It is worth noting that more than a third of all the published studies (Saunders et al. 2012; Roberts et al. 2013, 2015, 2018; Cole et al. 2014, 2015, 2016; Ellison et al. 2014; Neveux et al. 2016; Valero-Rodriguez et al. 2020) were carried out by the MACRO group at James Cook University (Australia), which worked specifically with *Oedogonium* species, consequently skewing the data. Nevertheless, *Oedogonium* has been consistently identified as a suitable candidate for wastewater treatment by other studies (Wang et al. 2013; Yun et al. 2014; Tabinda et al. 2019; Piotrowski et al. 2020; Lawton et al. 2021; Rearte et al. 2021; Kube et al. 2022; Liu et al. 2023). Genera not within the Chlorophyceae were also included in this review given that the aim of this work was to compare the results of suspended growth systems using algae growing as filaments. Depending on the authors, these are referred to either as macroalgae (Kube et al. 2022) or microalgae (Tan et al. 2018), the debate regarding which term is more appropriate is out of the scope of this review.

Across all studies, the biomass productivity (Fig. 4) was expressed either by volume (g L^{-1} day^{-1}) or by surface area (g m^{-2} day^{-1}). Apart from 4 studies, which did not report the productivity (Wang et al. 2013; Ellison et al. 2014; Tan et al. 2018; Tabinda et al. 2019), the results ranged from 0.11 g DW $m^{-2}day^{-1}$ for a *Spirogyra* culture treating municipal WW (Ge et al. 2018) to 40 g DW m^{-2} day^{-1} for a *Spirogyra* pond treating water from contaminated rural streams amended with nitrogen and phosphorus (Kim et al. 2018). As can be seen in Fig. 4, *Oedogonium* is not only the genus

Table 2 Studies using filamentous algae to treat wastewater, where: DW, dry weight; AFDW, ash free dry weight; TN, total nitrogen; TP, total phosphorus; COD, chemical oxygen demand; DRP, dissolved reactive phosphorus; DIN, dissolved inorganic nitrogen; DIC, dissolved inorganic carbon and NH_4-N, nitrogen as ammonium

Wastewater	Genera	Productivity (DW)	TN_i - TN_f (% Removal)	TP_i - TP_f (% Removal)	COD_i - COD_f (% Removal)	Other contaminants removed	Pathogen removal	Biochemical analysis of biomass	Reference
ABS industry effluent	*Tribonema*	0.267 g L^{-1} day^{-1}	40 - 4 (90)	12 - 0.1 (99)	288 - 51.8 (82)	Organic pollutants	Not assessed	Yes	Ali Kubar et al. (2022)
Aquaculture effluent	*Oedogonium*	3.8 to 23.8 g m^{-2} day^{-1}	2.3 - 0.9 (61) without CO_2	0.35 - 0.29 (16) without CO_2	-	-	-	Yes	Cole et al. (2014)
Aquaculture effluent	*Oedogonium*	23.9 to 35.7 g m^{-2} day^{-1}	2.6 - 0.03 (99)	-	-	Trace elements	Not assessed	Yes	Cole et al. (2015)
Ash water	*Oedogonium*	6.8 to 22.5 g m^{-2} day^{-1}	-	-	-	Trace elements	-	-	Roberts et al. (2013)
Ash water	*Oedogonium*	2.9 to 8.2 g m^{-2} day^{-1}	-	-	-	Al and Zn below regulatory level	-	Yes	Roberts et al. (2015)
Ash water	*Hydrodiction, Rhizoclonium, Oedogonium*	0.040 to 0.059 g L^{-1} day^{-1}	-	-	-	Trace elements	-	-	Saunders et al. (2012)
Ash water	*Oedogonium*	-	-	-	-	Trace elements	-	-	Ellison et al. (2014)
Contaminated rural streams	*Spirogyra*	40 g m^{-2} day^{-1}	2.85 - 0.6 (80)	0.31 - 0.06 (81)	No removal	-	Not assessed	-	Kim et al. (2018)
Municipal	*Oedogonium*	3.03 to 15.31 g m^{-2} day^{-1}	-	-	-	-	Not assessed	Yes	Piotrowski et al. (2020)
Municipal	*Oedogonium, Klebsormidium*	4.8 to 8.1 g m^{-2} day^{-1} AFDW	5.3 - 0.05 (99) DIN	2.6 - 1.3 (50) DRP	-	-	Not assessed	Yes	Lawton et al. (2021)
Municipal	*Oedogonium*	6.3 to 9.2 g m^{-2} day^{-1}	-	-	-	-	Not assessed	Yes	Neveux et al. (2016)
Municipal	*Oedogonium*	8.9 to 15.8 g m^{-2} day^{-1}	3.18 - 2.4 (36)	0.92 - 0.3 (65)	-	Trace elements	-	Yes	Cole et al. (2016)
Municipal	*Hydrodiction*	4.34 to 5.81 g m^{-2} day^{-1}	9.9 - 3.9 (60)	0.6 - 0.15 (74)	-	-	Not assessed	-	Min et al. (2019)
Municipal	*Oedogonium, Klebsormidium*	4.1 to 8.2 g m^{-2} day^{-1} AFDW	19.5 - 2.9 (85) DIN	4.3 - 1.7 (60) DRP	-	-	Not assessed	Yes	Lawton et al. (2021)
Municipal	*Oedogonium*	0.102 g L^{-1} day^{-1}	23.75 - 7.1 (70) TDN	6.77 - 0.7 (90)	27.5 - 0.3 (99) DIC	-	Not assessed	-	Kube et al. (2022)
Municipal	*Oedogonium, Spirogyra, Ulothrix, Vaucheria*	3.37 g m^{-2} day^{-1}	-	-	-	-	Not assessed	Yes	Yun et al. (2014)
Municipal	*Spirogyra*	2.17 to 2.9 g m^{-2} day^{-1}	10.7 - 0.9 (91.1)	1.47 - 0.7 (51.2)	-	-	-	Yes	Ge et al. (2018)
Municipal	*Spirogyra*	2.92 to 4.03 g m^{-2} day^{-1}	10.7 - 0.4 (96)	1.47 - 0.6 (60)	-	-	-	Yes	Ge et al. (2018)

Table 2 (continued)

Wastewater	Genera	Productivity (DW)	TN_i - TN_f (% Removal)	TP_i - TP_f (% Removal)	COD_i - COD_f (% Removal)	Other contaminants removed	Pathogen removal	Biochemical analysis of biomass	Reference
Municipal	*Spirogyra*	0.11 to 0.40 g m^{-2} day^{-1}	16.06 - 7.2 (55)	0.63 - 0.2 (69)	-	-	-	Yes	Ge et al. (2018)
Municipal	*Oedogonium*	12.7 to 13.8 g m^{-2} day^{-1}	0.65 g $N.m^{-2}.d^{-1}$	-	-	-	Not assessed	Yes	Neveux et al. (2016)
Municipal	*Oedogonium*	6.8 to 9.9 g m^{-2} day^{-1}	41.2 - 15.7 (62)	6.7 - 1.7 (75)	53 - 22.8 (57)	Trace elements	Yes	Yes	Neveux et al. (2016)
Piggery effluent	*Hydrodiction, Oedogonium, Anabaena, Spirulina*	-	65.5 - 2.6 (96)	6.2 - 0.4 (93)	90.7 - 34 (63)	-	-	-	Wang et al (2013)
Piggery effluent	*Stigeoclonium*	-	486 - 73 (85)	149 - 37.3 (75)	1,474 - 310 (79)	-	Not assessed	-	Tan et al. (2018)
Slaughterhouse effluent	*Stigeoclonium, Oedogonium*	0.45 g L^{-1} day^{-1}	61 - 3 (96) NH_4-N	5.9 - 0.5 (92) DRP	-	-	Not assessed	Yes	Rearte et al. (2021)
Synthetic growth medium	*Oedogonium, Stigeoclonium, Hyalotheca*	1 to 11 g m^{-2} day^{-1}	-	-	-	-	-	-	Valero-Rodriguez et al. (2020)
Synthetic growth medium	*Oedogonium*	1.13 g m^{-2} day^{-1}	-	-	-	-	-	-	Liu et al. (2023)
Textile industry effluent	*Oedogonium, macrophytes*	-	-	-	1,140 - 6.4 (99)	Trace elements	Not assessed	-	Tabinda et al. (2019)
Water treatment residuals	*Oedogonium*	10.3 to 13.6 g m^{-2} day^{-1}	-	-	-	74 % Al	Not assessed	-	Roberts et al. (2018)

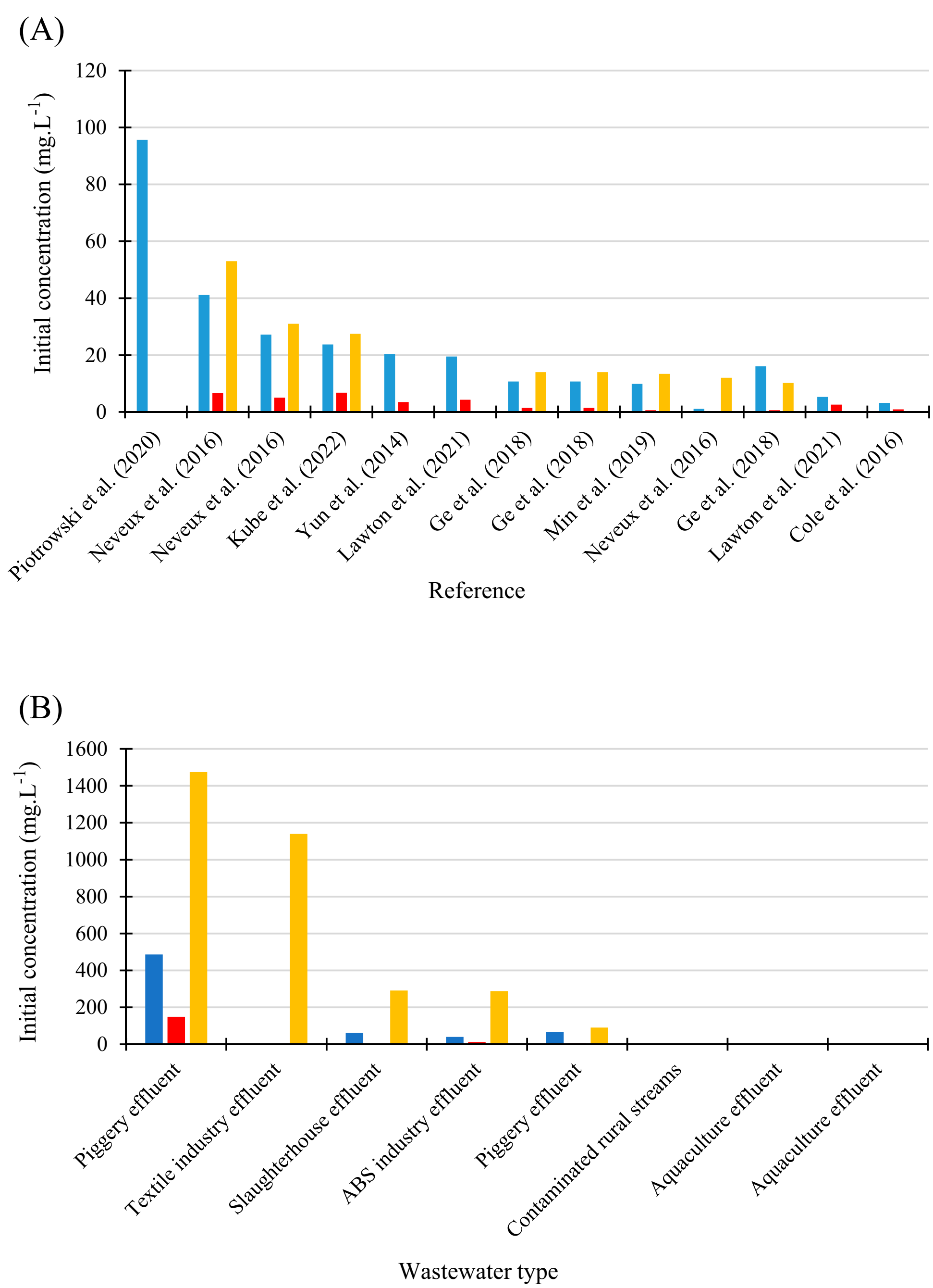

Fig. 1 Concentration of chemical oxygen demand (■), total phosphorus (■) and total nitrogen (■) in effluents used for the evaluation of the wastewater treatment performance of filamentous algae, **a**) those using municipal WW, the x-axis shows the reference of each study; (**b**) those conducted with industrial WW, the x-axis shows the wastewater type

with the most reported productivities, but also the one with the highest variability in productivity. This is understandable given that this genus has been tested with wastewater from different sources, at varied water exchange rates, and in a significant range of PBRs, from 1 L indoor flasks to 27,000 L outdoor tanks and over a broad range of light intensities as can be observed on Table 3. It is also a cosmopolitan genus with a high number of reported species, which grow in different environments and has a variety of life habits (Lawton et al. 2014).

Specifically, in the algal WWT field the relevance of the productivity lies in its relationship with carbon, nitrogen and phosphorus removal (Rearte et al. 2021), however, it cannot be used as a performance indicator in isolation. The effectiveness of the nutrient uptake and pollutant sequestration is not always directly proportional to the biomass growth. For example, Neveux et al., (2016) reported 62 % of TN and 75 % of TP removal on municipal WW using *Oedogonium* cultures with productivities ranging from 6.8 to 9.9 g m^{-2} day^{-1} (Neveux et al. 2016). In contrast, Cole et al., (2016) reported 36.1 % of TN and 64.6 % of

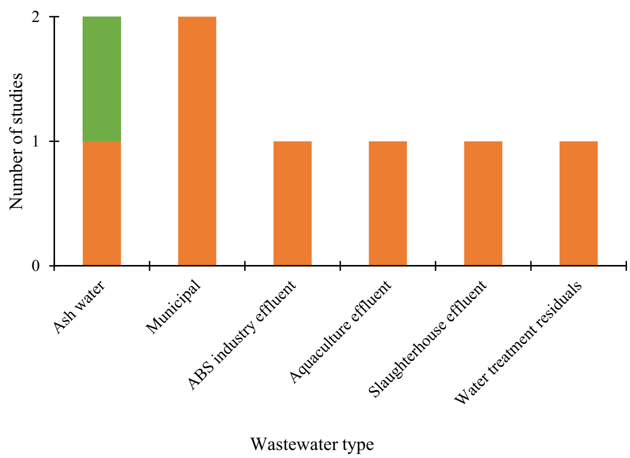

Fig. 2 Filamentous algae wastewater treatment studies sparging CO_2 enriched air and the CO_2 source, the y-axis shows the number of studies performed with each WW, the x-axis the type of WW. Studies performed using purified CO_2 sparging (■) and studies performed using CO_2 from flue gases (■)

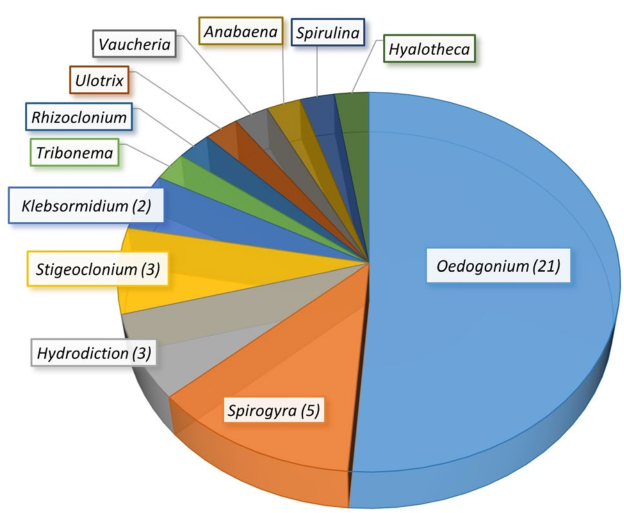

Fig. 3 Genera included in wastewater treatment research; the number of studies (>1) incorporating a specific genus are indicated in parentheses

TP removal from another municipal WW using the same genus, while reporting higher biomass yields of between 8.9 and 15.8 g m^{-2} day^{-1} . Perhaps, more surprising was that (Neveux et al. 2016) had less pre-treatment and almost one order of magnitude higher nutrient concentration than reported by Cole et al. (2016).

There was a wide range of results regarding the removal of nitrogen, which was reported only by 17 of the 28 reviewed studies. In seven cases the authors reported removals equal or greater than 90 % (Wang et al. 2013; Cole et al. 2015; Ge et al. 2018; Lawton et al. 2021; Rearte et al. 2021; Ali Kubar et al. 2022) and the lowest nitrogen removal (36.1 %) was reported for an *Oedogonium* culture treating municipal WW (Cole et al. 2016). Phosphorus removal was only reported by half of the studies. In five cases the removal was 90 % or higher (Wang et al. 2013; Ge et al. 2018; Rearte et al. 2021; Ali Kubar et al. 2022; Kube et al. 2022), and the lowest removal (15.7 %) was reported for an *Oedogonium* culture growing in synthetic medium without CO_2 injection (Cole et al. 2014). Finally, the chemical oxygen demand removal was reported only by one quarter of the studies (Wang et al. 2013; Neveux et al. 2016; Ge et al. 2018; Kim et al. 2018; Tan et al. 2018; Tabinda et al. 2019; Ali Kubar et al. 2022; Kube et al. 2022), with results ranging from 99–98 % of removal (Tabinda et al. 2019; Kube et al. 2022) to no detectable removal reported for a *Spirogyra* culture growing in municipal effluent (Ge et al. 2018) or amended contaminated rural stream water (Kim et al. 2018).

One of the perceived advantages of filamentous algal cultures versus unicellular algal cultures is the ease of separating the biomass from the media once the treatment is completed. Considering biomass separation, 11 studies reported the biomass was separated from the treated effluent by filtration or retention of the biomass with mesh screens (Cole et al. 2014, 2015, 2016; Neveux et al. 2016; Min et al. 2019; Lawton et al. 2021; Rearte et al. 2021; Kube et al. 2022). The pore or mesh sizes ranged from 85 µm (Kube et al. 2022) to 750 µm (Cole et al. 2014, 2015), most separated *Oedogonium* cultures. An interesting adaptation was reported (Cole et al. 2016) where, to prevent the mesh screens from getting clogged, a tube for air sparging was installed just below the outlet preventing the accumulation of biomass. Surprisingly, the remaining studies did not report on how to remove the algae from the treated wastewater. This is a crucial aspect that requires more consideration for the future transferability of the technology to the industry.

Seven studies used filamentous algae alone or combined with submerged plants to treat effluents with organic or metal contamination. Four of those studies reported a reduction of trace element concentrations in ash water from the washing of flue gases from a coal power plant in Australia (Saunders et al. 2012; Roberts et al. 2013, 2015; Ellison et al. 2014). In these studies, the remediation was performed mostly by *Oedogonium* cultures, and also *Hydrodiction* and *Rhizoclonium* (Saunders et al. 2012). Two other studies reported metal removal from effluents from a textile industry using a combination of macrophytes and *Oedogonium* (Tabinda et al. 2019) and within a water treatment pond carried out by *Oedogonium* (Roberts et al. 2018). Ali Kubar et al. (2022) also reported acrylonitrile butadiene styrene removal from an industrial WW using *Tribonema* cultures. Surprisingly, three cases (Yun et al. 2014; Neveux et al. 2016; Piotrowski

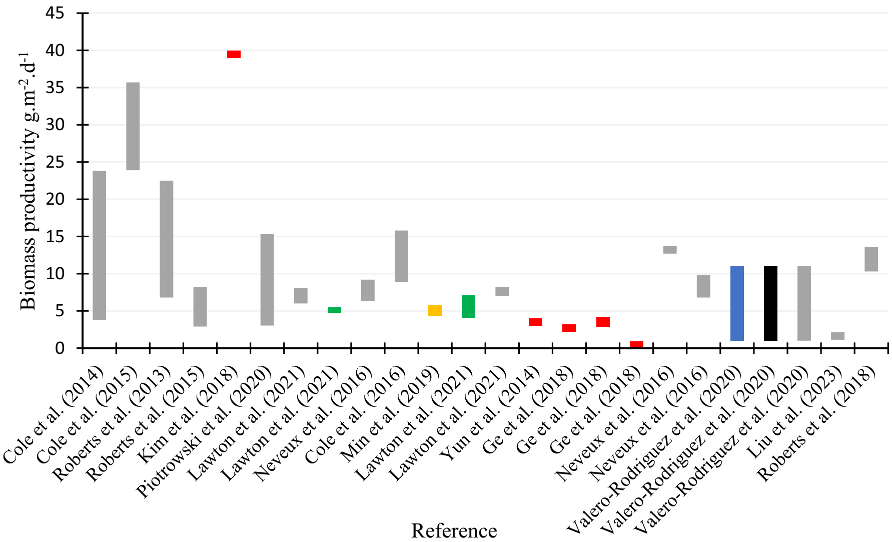

Fig. 4 Reported ranges of productivity. The study references are shown on the x-axis, and the productivity values (g DW m^{-2} day^{-1}) on the y-axis. Bars filling indicate the genera used in the studies as follows: *Hyalotheca* (■), *Hydrodiction* (■), *Klebsormidium* (■), *Oedogonium* (■), *Spirogyra* (■) and *Stigeoclonium* (■)

et al. 2020) reported no data regarding nutrient or metal removal on effluents with organic load.

Interestingly, 16 out of 28 studies performed analysis of the biochemical composition of the recovered algal biomass. The proposed application for the produced biomass was mostly as biofuel feedstock (Cole et al. 2014; Yun et al. 2014; Neveux et al. 2016; Ge et al. 2018; Lawton et al. 2021; Rearte et al. 2021; Ali Kubar et al. 2022), bioethanol in the cases where carbohydrates were predominant and biodiesel when the fatty acids composed the biomass main energy reserve. There were alternative uses such as livestock feed, fertilizers and cellulose extraction.

Increasingly, treated wastewater is reused for agricultural irrigation (Guardiola-Claramonte et al. 2012). From a public health perspective, the pathogen load of the treated effluent is a key aspect in reducing human exposure to harmful microorganisms, one that was surprisingly overlooked in most studies. Only one study out of 13 using municipal WW measured reduction of the faecal indicator *Escherichia coli* (Neveux et al. 2016), where *Oedogonium* ponds treating municipal WW achieved a 3 log reduction during a summer study.

Environmental conditions

The main advantage of using algal ponds to treat effluents comes from the ability of these organisms to utilise solar energy via photosynthesis to grow and absorb carbon, nitrogen, phosphorus and produce dissolved oxygen for heterotrophic respiration of organic carbon. The main energetic input of these systems is photosynthetically active radiation (PAR; 400-700 nm). This variable is key, both for the performance comparison of different cultures and as a design variable, given that it reflects the energy availability for a treatment system. To allow a simple review of the different reported conditions we calculated the average daily photon flux from maximum and lower values of PAR radiation reported by the studies (Table 3).

Of 28 studies considered in this review, one indoor (Ali Kubar et al. 2022) and five outdoor (Roberts et al. 2015, 2018; Kim et al. 2018; Tabinda et al. 2019; Piotrowski et al. 2020) experiments do not have PAR received by the cultures reported, thus the remaining 22 studies were compared (Fig. 5) by the PAR received. All the outdoor cultures in the reviewed studies shown in Fig. 5 took place in Townsville, Australia Latitude S 19.26° (Roberts et al. 2013; Cole et al. 2014, 2015, 2016; Neveux et al. 2016). In comparison, the greenhouse cultures (i.e. receiving sunlight) were carried out in Ansan, South Korea Latitude N 37.32° (Min et al. 2019); Te Puke, New Zealand Latitude S 37.78° (Lawton et al. 2021) and Rotorua, New Zealand Latitude S 38.14° (Lawton et al. 2021). Surprisingly, the indoor experiment conducted by Yun et al. (2014) was the one to report the highest photon flux with 65.7 mol photons m^{-2} day^{-1}. The experiments performed outdoors or in greenhouses had average PAR radiation values 10-fold higher than those conducted indoors (Fig. 5). Also six of the indoor studies (Saunders et al. 2012; Wang et al. 2013; Ellison et al. 2014; Tan et al. 2018; Valero-Rodriguez et al. 2020; Liu et al. 2023) received an average photon flux of 5 mol photons m^{-2} day^{-1} or less, which is arguably low.

However, this is only one aspect of the radiation phenomenon. The light attenuation effect of algal biomass, the geometry and depth of the PBRs play a crucial role in the effective light distribution through the water column. Rearte et al. (2021) reported 68.5 µmol photons m^{-2} s^{-1} of average irradiance inside 6 cm diameter columnar PBRs when the average incident irradiance was 300 µmol photons m^{-2} s^{-1}. In contrast, Min et al. (2019), reported a mean irradiance of 49.3 µmol photons m^{-2} s^{-1} inside a 30 cm deep raceway pond receiving a mean incident irradiance of 633 µmol photons $m^{-2} s^{-1}$. Unfortunately, most studies lack this information, which would allow for a comparison of the irradiation received per unit of treating volume.

A unique study conducted by Min et al. (2019) at Konkuk University, South Korea used a raceway pond with submerged lighting. The experiment compared the performance of a filamentous green algae culture illuminated with solar radiation alone versus one sunlight irradiated culture supplemented with artificial light from LED lamps. Although the treatment effect increased more than 3-fold with the underwater light system, it should be noted that the initial concentrations of TN and TP were low at the HRAP's inlet (less than 10 and 1 mg L^{-1} respectively); the effluent was autoclaved to eliminate bacterial activity and the raceway was constructed inside a greenhouse. Hence, to achieve the enhanced performance, covered algal ponds with underwater lighting must be constructed after conventional WWTPs. This might be an interesting solution for urban situations where the surface availability is a constraint, allowing larger volumes of treatment per square metre. However, the CAPEX of a potential WWTP with this technology is likely higher, which may not be an accessible solution to WW treatment in small rural and regional communities.

The size of the cultures is another key aspect of the experimental design given that, generally, larger volumes tend to reflect more the intricate and complex interactions occurring within the cultures. The same features that make these mixes of phototrophic organisms, aerobic bacteria, micro arthropods, and other organisms difficult to model (Rossi et al. 2020) may also make them resilient to environmental fluctuations. Liu et al. (2020) present a scheme which explores the intricate interactions between the heterogeneous groups that take part in these systems. The larger volume, which gives cultures potential buffering capacity for environmental variations has the drawback that it is often harder to perform experiments, both because of the research resources and the logistics.

Figure 6(A) and (B) show the distribution of size and duration of studies included in this review. As might be expected, most of the studies were performed with smaller pond sizes (≤ 100L). More than half of the studies were carried out with culture volumes smaller than 10 L (Saunders et al. 2012; Wang et al. 2013; Ellison et al. 2014; Yun et al. 2014; Ge et al. 2018; Tan et al. 2018; Tabinda et al. 2019; Valero-Rodriguez et al. 2020; Lawton et al. 2021; Rearte et al. 2021; Ali Kubar et al. 2022; Kube et al. 2022; Liu et al. 2023). Apart from the experiment above by Min et al., (2019), there were two studies performed in greenhouses with 4 L cultures (Lawton et al. 2021). The biggest variability in culture volumes was identified in studies with outdoor cultures, where the smallest had only 8 L (Tabinda et al. 2019) and the largest 27,000 L (Cole et al. 2016).

Regarding the experimental time frames, half of the studies had a duration of up to 14 days. Only 10 out of 28 experiments had a duration of more than four weeks. The remarkable exceptions were a study conducted in Townsville, Australia (Cole et al. 2016), where the researchers evaluated the performance of the system for one year, and a study performed in Ansan, South Korea (Kim et al. 2018), which lasted 210 days. The treatment performance of a short duration algal culture in WW might not reflect a process that these systems are able to sustain over long periods. Natural evolution gave the algae strategies to survive difficult and changing environments, endure drought periods and flooding with the related variations in conductivity, nutrient levels, pH and radiation (Evans, 1959). Studies measuring the algal performance for short time periods risk having results that are explained more by a survival state of these organisms rather than a metabolic process sustainable in time. Imbalanced availability of nitrogen, phosphorus, carbon or lack of micronutrients can be overcome over time but might be the cause of sudden or progressive algal growth decline in longer experiments (e.g. months). Some experiments had results that do not clearly show whether the cultures reached a stable point, and in certain cases the productivity even seemed to have decreasing trends (Roberts et al. 2013).

Also, longer duration studies better describe the treatment performance under changing seasonal conditions. To design reliable systems delivering treatment year-round, the effect of the light cycle and temperature variation must be assessed at some point of the project, especially in non-tropical regions where the winter sunlight can be less than half of that received in summer. There is a lack of studies exploring the shorter daylight seasons, only 5 were performed in autumn weeks and 2 across winter weeks. Only in 2 of those cases was the algal treatment process assessed in non-tropical sites. It is evident that there is a need to explore the performance of filamentous algal systems, not only across the seasons but also in regions where the climatic conditions are more variable during the year. It is recommended that the final WWTP design and validation are conducted during the local winter, which is normally the worst-case scenario for the performance of natural treatment systems.

There is little evidence of an increase in culture size (volume) over the 13 years that this review encompasses (Fig. 7), suggesting few small-scale systems (≤ 100 L) translated to larger applications and that there was not an increase in experiments using large systems. There are some exceptions though, assuming publication occurred not long after the experiments were performed, some scale up of experiments can be identified from 2012 to 2015 and in 2016. In 2015 to 2016, a series of studies were conducted aiming to treat ash water from a coal fired power plant (Saunders et al. 2012;

Table 3 Environmental conditions and culture management parameters during the studies, where PBR: photo bioreactors; HRT: hydraulic retention time; nr: not reported

Wastewater	Site	Local Season (Latitude in degrees)	Photon flux mean (mol photons m^{-2} day^{-1})	Setting	Culture volume (L)	PBR type	Operation mode	HRT (days)	Duration (weeks)	Reference
ABS industry effluent		-	nr	Indoor	0.9	Cube	Batch	-	1.3	Ali Kubar et al. (2022)
Aquaculture effluent	Townsville, Australia	Autumn (19.33 S)	23.2	Outdoor	853	Aerated tanks	Semicontinuous	10 - 0.2	0.6	Cole et al. (2014)
Aquaculture effluent	Townsville, Australia	Spring (19.33 S)	49.3	Outdoor	864	Aerated tanks	Semicontinuous	1	1	Cole et al. (2015)
Ash water	Townsville, Australia	Spring (19.33 S)	18.5	Outdoor	60	Aerated tanks	Semicontinuous	nr	4	Roberts et al. (2013)
Ash water	Tarong, Australia	Spring (19.33 S)	nr	Outdoor	15,000	Aerated tanks	Semicontinuous	nr	7	Roberts et al. (2015)
Ash water		-	3.8	Indoor	1	Glass bottle	Semicontinuous	nr	2.1	Saunders et al. (2012)
Ash water		-	4.3	Indoor	1	Glass bottle	Semicontinuous	nr	3	Ellison et al. (2014)
Contaminated rural streams	Ansan, South Korea	Spring-Summer-Autumn (37.32 N)	nr	Outdoor	6,000	Shallow channel	Semicontinuous	4	30	Kim et al. (2018)
Municipal	Madison, USA	Summer-Autumn (43.11 N)	nr	Outdoor	495	Aerated tanks	Batch	-	1.3	Piotrowski et al. (2020)
Municipal	Rotorua, New Zealand	Summer (38.14 S)	18.8	Greenhouse	4	Buckets	Semicontinuous	4	1.7	Lawton et al. (2021)
Municipal	Townsville, Australia	Summer (19.33 S)	48.1	Outdoor	20	Buckets	Semicontinuous	20 - 5	2	Neveux et al. (2016)
Municipal	Townsville, Australia	Summer-Autumn-Winter-Spring (19.33 S)	32.3	Outdoor	27,000	Aerated tanks	Semicontinuous	1	52	Cole et al. (2016)
Municipal	Ansan, South Korea	nr (37.32 N)	27.35	Greenhouse	8,000	Raceway pond	Semicontinuous	8	4.6	Min et al. (2019)
Municipal	Te Puke, New Zealand	Summer (37.79 S)	16.9	Greenhouse	4	Buckets	Semicontinuous	4	1.7	Lawton et al. (2021)
Municipal		-	15.6	Indoor	0.35	Erlenmeyer flasks	Semicontinuous	0,5	4.3	Kube et al. (2022)
Municipal		-	65.7	Indoor	5	Shallow trays	Semicontinuous	50 - 2	2	Yun et al. (2014)
Municipal		-	8.45	Indoor	0.8	Columns	Batch	-	2	Ge et al. (2018)
Municipal		-	5.8	Indoor	4	Aquariums	Semicontinuous	14	6	Ge et al. (2018)
Municipal		-	5.8	Indoor	4	Aquariums	Semicontinuous	14	6	Ge et al. (2018)
Municipal	Townsville, Australia	Summer (19.33 S)	48.1	Outdoor	20	Buckets	Semicontinuous	20 - 5	2	Neveux et al. (2016)
Municipal	Townsville, Australia	Winter (19.33 S)	25.1	Outdoor	10,000	Aerated tanks	Semicontinuous	20	8	Neveux et al. (2016)
Piggery effluent		-	2	Indoor	1	Erlenmeyer flasks	Batch	-	1	Wang et al (2013)

Table 3 (continued)

Wastewater	Site	Local Season (Latitude in degrees)	Photon flux mean (mol photons m^{-2} day^{-1})	Setting	Culture volume (L)	PBR type	Operation mode	HRT (days)	Duration (weeks)	Reference
Piggery effluent		-	1.8	Indoor	0.1	Erlenmeyer flasks	Batch	-	2	Tan et al. (2018)
Slaughterhouse effluent		-	13.7	Indoor	0.8	Columns	Semicontinuous	5 - 1.7	1.4	Rearte et al. (2021)
Synthetic growth medium		-	2.18	Indoor	1	Glass bottle	Semicontinuous	nr	5	Valero-Rodriguez et al. (2020)
Synthetic growth medium		-	5	Indoor	0.5	Rectangular containers	Batch	-	3	Liu et al. (2023)
Textile industry effluent	Lahore, Pakistan	nr (31.52 N)	nr	Outdoor	8	Buckets	Batch	-	1	Tabinda et al. (2019)
Water treatment residuals	Townsville, Australia	Summer-Autumn (19.33 S)	nr	Outdoor	20	Buckets	Semicontinuous	7	12	Roberts et al. (2018)

Roberts et al. 2013, 2015; Ellison et al. 2014). The progression started from 1 L glass bottles indoors to 15,000 L aerated tanks outdoors. In another case in 2016, the researchers started testing culture conditions and performance on municipal WW in 20 L buckets (Neveux et al. 2016), progressed to 10,000 L tanks and finally experimented with 27,000 L ponds (Cole et al. 2016). Finally, Cole et al. (2014, 2015) published two studies where biomass production was assessed coupled with final treatment of fishery effluent. Although these two studies were conducted using similar volume ponds, the first (Cole et al. 2014) had more interventions to the influent (CO_2 sparging and nutrient amendment), the second (Cole et al. 2015) had fewer interventions but a longer duration.

The operation mode of the cultures is another condition to consider because it directly impacts the transferability of these treatment processes to large scale alternatives in real-life scenarios. The operation of the studies reviewed in this article were classified as batch and semi-continuous. The batch mode means the inoculum and the wastewater were put in the PBR at the beginning of the run and the culture can be monitored during the process, but no other input occurs until the end of the experiment (Lim & Shin 2013). Alternatively, the semi-continuous mode implies that there is an input stream of medium and an exchange of medium with or without the algae. It is referred to as "semi" because the medium exchange is normally not constant but performed in pulses during the run, but there are some exemptions. The goal of the semi-continuous mode is to reach a steady state of algal growth/nutrient removal by balancing the inlet and outlet streams.

Three quarters of all the experiments were operated in semi-continuous mode, which arguably better reflects the operational mode for utilities treating wastewater than the batch mode. The different hydraulic retention times tested on these studies range from 4.8 h to 15 days, which cover a wide range of scenarios. Only seven out of all the compiled experiments were operated in batch mode, generally with the aim of exploring the nutrient removal dynamics of the cultures.

Another aspect that must be considered is that filamentous algae, as most organisms, must acclimate upon facing new environmental conditions, even if these can support the culture's growth. One acclimation method that proved to give good results was to progressively exchange the stock culture media with the target WW (Neveux et al. 2016; Rearte et al. 2021).

Finally, regarding the types of photo bioreactors, the most used containers were buckets and aerated tanks. The first option was used in seven of the reported studies (Neveux et al. 2016; Roberts et al. 2018; Tabinda et al. 2019; Lawton et al. 2021), all performed with natural sunlight either in a glasshouse or outdoors and using volumes between 4 and

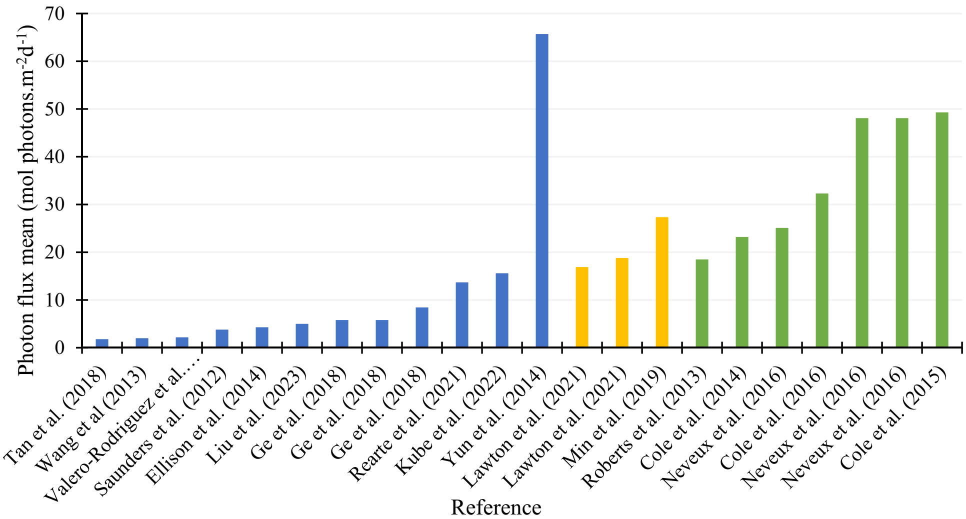

Fig. 5 Average irradiance received by wastewater cultures of filamentous algae**.** The study references are shown on the x-axis and the reported mean photon flux (PAR) on the y-axis. Indoor studies (■), greenhouse studies (■) and outdoor studies (■)

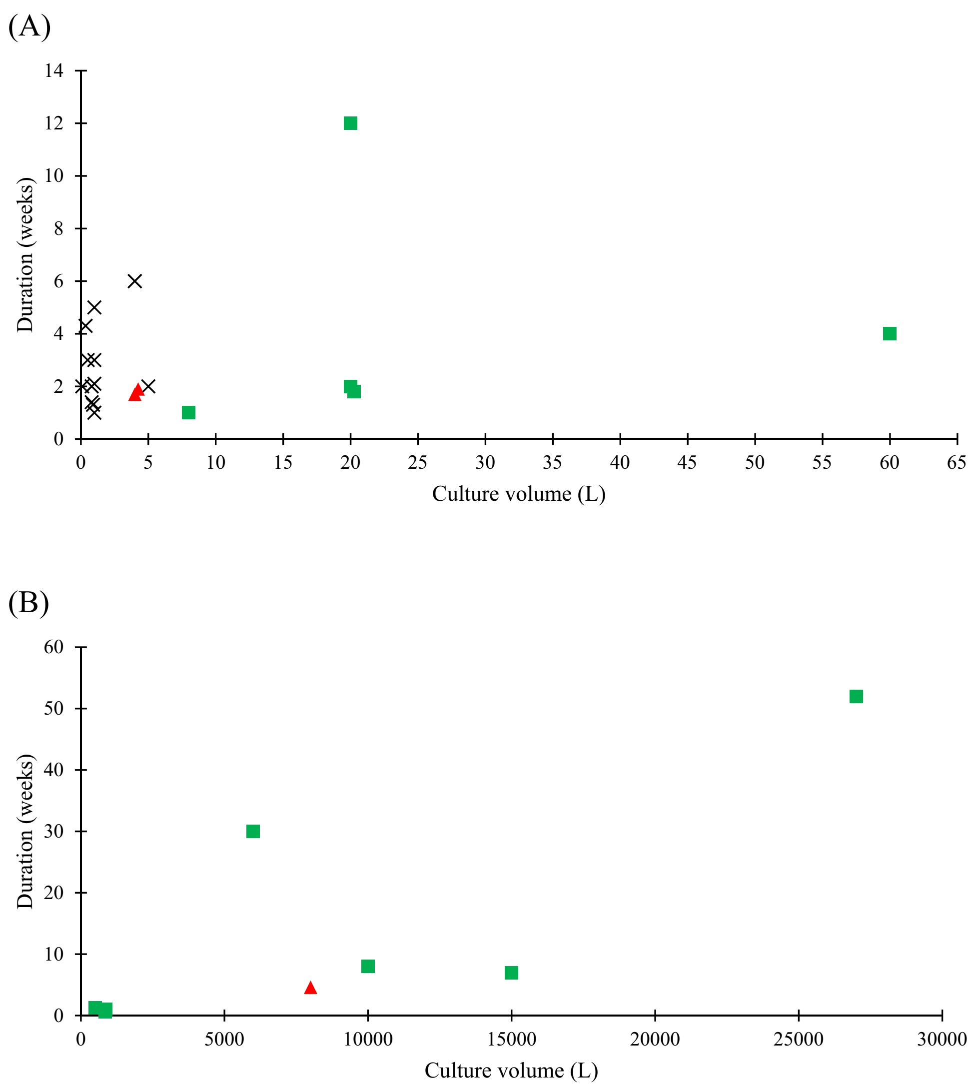

Fig. 6 Culture volume and experimental duration, (**A**) conducted with cultures ≤100 L, and (**B**) cultures > 100 L. Culture volumes (L) are shown on the x-axis, duration in weeks is shown on the y-axis. Studies carried out indoors (×) inside greenhouses (▲) and outdoors (■)

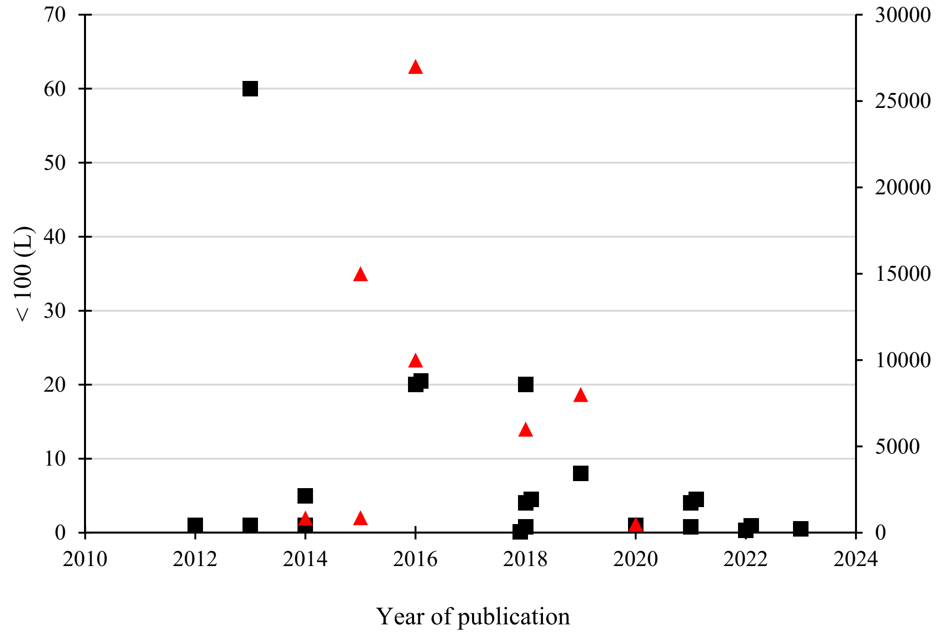

Fig. 7 Experimental culture volumes for studies reported 2012 -2022. The left y-axis indicates the volume of the cultures < 100 L (■), the right y-axis indicates the volume of the cultures > 100 L (▲), and the x-axis indicates the year that these studies were published

20 L. The aerated tanks were used in seven cases in outdoor settings with culture volumes between 60 and 27000 L (Roberts et al. 2013, 2015; Cole et al. 2014, 2015, 2016; Neveux et al. 2016; Piotrowski et al. 2020). The rest of the studies used a variety of laboratory containers, such as Erlenmeyer flasks, clear acrylic columns, cubic flasks and glass bottles, or models of raceway ponds.

Discussion

From a phycological point of view, even if this is a niche within the algal wastewater treatment field, which has seen publications of experimental studies for only 13 years, there has been a worthy exploration of many filamentous genera. Bioprospecting has consistently identified *Oedogonium* spp., *Spirogyra* spp., *Hydrodiction* spp. and *Stigeoclonium* spp. as suitable candidates for wastewater treatment as they are the three most used genera in the studies reviewed here.

It is surprising that there are more studies exploring filamentous algal biomass productivity and its biochemical quality than analysing the nitrogen or phosphorus removal from the wastewater. Furthermore, only one study assessed pathogen removal (Neveux et al. 2016). This suggests that there is more interest in producing biomass and the possible derived bio-compounds, than the performance regarding wastewater treatment to protect public health. Even if both processes are coupled, the system will not always promote them equally. Depending on design parameters, the algal strains, wastewater types, retention times and types of PBRs, algal ponds can be constructed with a spectrum of goals. We could frame that spectrum between two approaches. Firstly, using wastewater to produce biomass, where the effluent is just regarded as a free source of nutrients for algal growth. In this case the wastewater treatment is a secondary effect that adds attractiveness to the system given its environmental benefit. Interrogating the reported productivities in the reviewed literature it becomes apparent that there is a likely bottleneck for the biomass production approach. If a system can sustain a productivity of 20 g m^{-2} day^{-1}, which only four studies in this review attained (Roberts et al. 2013; Cole et al. 2014, 2015; Kim et al. 2018), a 1 ha pond would produce 200 kg of dry biomass per day. This implies a limitation on the possible economical exploitation, using a biorefinery approach, able to access only 73 t of raw material per year. Secondly, treating wastewater with algal ponds, where the main goal is to improve the effluent quality for discharge, and the generated biomass is regarded just as a by-product together with treated wastewater for reuse. Taking these aspects into consideration, the utilisation of filamentous algae may be more readily adopted to satisfy an environmental need for wastewater treatment, producing an increasingly valuable product of treated wastewater. A commodity that is expected to be progressively scarcer in some regions due to climate change.

Having considered most of the factors playing a role in filamentous algal bioreactors performance, attempting to find relationships is desirable. Figure 8 (A) shows the studies, which reported biomass areal productivity, the wastewater initial nitrogen concentration and nitrogen removal. The first observation is that there is a lack of information meeting the requirements for this kind of comparison. There are too few data points to elaborate conclusions about different genera's

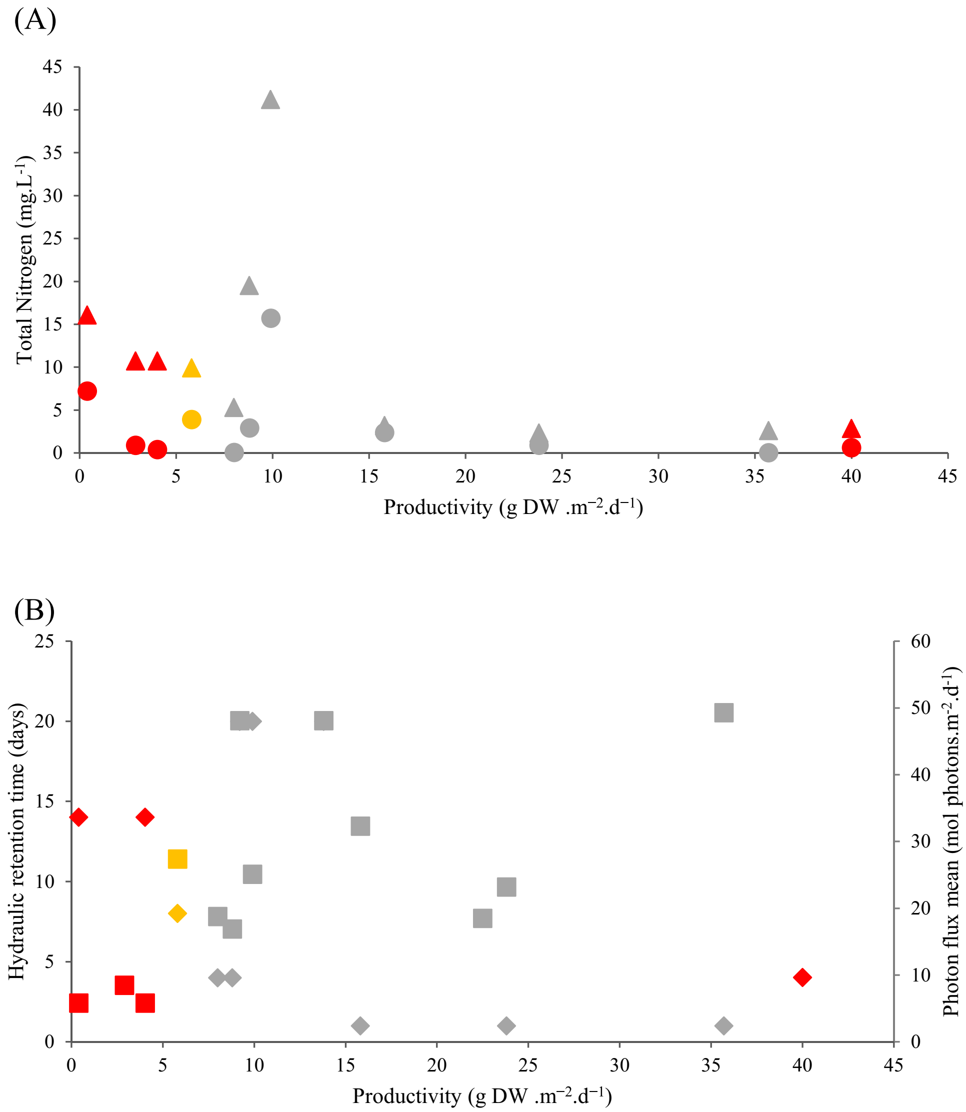

Fig. 8 Reported biomass productivity compared to (**A**) wastewater total nitrogen concentration before the algal pond (▲) and after the treatment (●). The y-axis indicates the TN concentration, and the x-axis indicates the biomass productivity. (**B**) Biomass productivity compared to hydraulic retention time (◆) and photon flux (■), the right y-axis indicates the hydraulic retention time, the left y-axis indicates the photon flux and the x-axis indicates the biomass productivity. On both (A) and (B) the marker's colours indicate the genus of the algae *Spirogyra* (■), *Hydrodiction* (■) and *Oedogonium* (■)

performances, for example. However, it is possible to conclude the data does not show a correlation between the initial total nitrogen concentration of the influent and the biomass productivity, nor with nitrogen removal. A way of making sense of this data is looking at the HRT and photon flux (Fig 8 (B)), which also shows a lack of information to assess correlations. In spite of this, an incipient effect can be deduced from the variation in HRT, the shorter the retention time, the higher the productivity. A logical phenomenon given that accelerating the pond's medium exchange implies higher nutrient fluxes. Finally, the photon flux received by the ponds seems to have a share in the phenomenon, understandably, as this is the energy input of the algal-bacterial consortia.

Each case study has different wastewater types and scenarios. As can be noticed in the literature, researchers across the world have attempted to use filamentous algal ponds to improve the wastewater quality in diverse settings and address the industries or populations needs. A thoughtful analysis of the projects must consider the origin of the influents and the treatment goals of the algal systems. There is great potential for filamentous algal ponds treating secondary effluent, replacing the aerobic stage or more steps of the conventional treatment process, for a simple solar powered system that does not rely on consumables or long retention times. This is an area within the field that is still in the first steps, with the capacity of addressing the needs of small communities especially in rural areas for easy to operate WWTPs and the bonus of in situ water recycling.

In other cases, there is an interest in further improving the quality of tertiary or fully treated effluents, particularly before discharge into water bodies. Even when the N-P-C levels are considerably low, large volumes constantly discharged into rivers, freshwater lakes or marine bays can have a dramatic ecological impact over time, also deteriorating strategic economical resources. It can also be the case that an existing treatment scheme needs improvement or expand it's capacity due to population or industry growth. This would be a different opportunity for filamentous algal ponds to provide an accessible solution at a lower cost compared to other systems, particularly at sites where land occupation is not an issue.

Organic pollutants and heavy metals removal is a goal addressed by a minority of the published studies, and one where it is even more difficult to reach general conclusions given that the trade-off between employed resources and achieved goals changes from one scenario to the other.

Conclusion

The primary benefit of filamentous algae is most likely wastewater treatment, enabling nutrient removal and importantly improved low cost biosolids separation when compared to microalgal systems. The most used filamentous alga was *Oedogonium*. The review highlighted the importance of reporting the composition of the influent and treated wastewater to enable assessment of treatment performance. Increasing wastewater reuse also requires the composition analysis and performance include microbiological parameters of public health significance. Supplementing wastewater with nutrients and CO_2 or diluting influents to enable filamentous algal growth should be avoided since it is unlikely to be a practical or economic option acceptable to water utilities for large scale wastewater treatment. Many of the studies reviewed emphasised filamentous algal productivity and composition. Although the biomass produced may be considered a secondary benefit, filamentous algal productivity data suggests large scale wastewater treatment systems will be required to produce sufficient biomass for economic viability. The review clearly demonstrates that, except for the research at James Cook University, Australia, few studies have transitioned from laboratory to field scale application. To further the adoption of the technology it is recommended future research focus on the acclimation of filamentous algae to growth in wastewater, which has received as minimum prior intervention as possible, with the objective of rapidly transitioning from laboratory to outdoor experimentation at scale.

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Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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