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Recent reports on domestic wastewater treatment using microalgae cultivation: Towards a circular economy

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ABSTRACT

The conventional wastewater treatment process (WWTP) is under continuous improvement, mainly to reduce the final pollutant contents in the effluent. The circular economy concept is driving this industry towards the recovery of the carbon (C) and nutrient (nitrogen – N and phosphorus – P) loads as valuable products, also to answer issues related to their depletion in nature. In this context, microalgae cultivation on wastewater has shown great promise in both water purification and the recovery of nutrients through additional biomass production. The further direction of the algal biomass to anaerobic co-digestion with wastewater sludge promises biogas yield improvements directing more of the carbon to energy recovery. Recent 100+ publications are here reviewed with the objective of compiling and comparing data on the cultivation of microalgae on domestic wastewater drawn from different stages of the conventional WWTP. A wide range of reactor types and scales, microalgae and bacteria inocula, and operational conditions are included, focusing on carbon and nutrient removal performance and the extent of their recovery in the produced biomass. However, most studies do not provide enough data tracking N, P, and C contents to allow the determination of their distribution among the possible system outputs. Still, some studies quantify the importance of removal mechanisms such as volatilization and precipitation, as well as microalgae uptake. Tentative mass balances on the three keys elements are presented, using the data disclosed in these few studies, highlighting the usefulness of these calculations and the need for their inclusion in future studies.

1. Introduction

The increase of human population is leading to developments in many sectors such as the urban, agricultural, and industrial, to accommodate the also increasing human necessities. This fast growth is resulting in an increase of waste production that is causing environmental pollution as well as accelerated depletion of natural resources (Li et al., 2019; Mustafa et al., 2021; Sharma et al., 2022). Jones et al. (2021) estimated a worldwide yearly wastewater production of around 360 billion m^3, stressing the importance of the continuous improvement of the existing wastewater treatment processes.

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There has been a tendency for the implementation of increasingly strict legislation on the discharge of water after treatment into the natural receiving systems. This is due to the developments in the environmental sector leading to deeper studies of the impacts of different compounds in the ecosystems, even when present in minimal concentrations. In this context, eutrophication is one of the most studied environmental phenomena that raised the need for the limitation of nitrogen and phosphorus (nutrients) discharges (Richmond, 2004; Sukla et al., 2019).

Thus, the main purpose of the wastewater treatment processes is still the removal of pollutants to return the water to the aquatic environment without inducing harmful changes in the latter. However, the paradigm is changing towards the circular economy concept (Geremia et al., 2021; Grady et al., 2011; Gray, 2004; Guldhe et al., 2017; Metcalf & Eddy et al., 2003; Mohsenpour et al., 2021; Sharma et al., 2022; Zhang and Liu, 2022). Consequently, changes in conventional wastewater treatment processes, such as those involving activated-sludge, nitrification, and denitrification, are being considered in order to reduce energy consumption and recover nitrogen, instead of converting it and releasing it to the atmosphere (Li et al., 2019; Mohsenpour et al., 2021; Sharma et al., 2022).

Microalgae cultivation has been used in wastewater treatment for as long as 3000 years according to the US EPA (2011) through the pond-based technology. Nevertheless, in the last years there has been a gain in popularity on the use of this technology due to its particular ability to recover valuable products from the wastewater (Geremia et al., 2021; Guldhe et al., 2017; Mohsenpour et al., 2021). Microalgae cultivation processes may therefore be used in wastewater treatment to shift from a linear economy to a circular economy. This is specifically applicable in the biological cycles, whereby the nutrients can be recovered from the wastewater and reused for the production of compounds such as biomethane (renewable energy) and C–N–P fertilizers (re-entering the food chain), among other added-value bioproducts (Kusumo et al., 2022).

Many works have been published about the capacity of microalgae to remove different pollutants (especially nitrogen and phosphorus) from wastewaters. However, reports providing the detailed data necessary for performing mass balances on resource recovery and fate are scarce, namely for the macro-nutrients carbon, nitrogen and phosphorus (e.g., carbon transformation is most often expressed in oxygen demand units, introducing further uncertainty into mass balances). This is due to the diverse pathways, physical, chemical and biochemical, through which these nutrients can be converted in open microalgae–bacteria systems. Nutrients can be recovered or lost in the solid (e.g., biosludge, inorganic precipitates), liquid (e.g., residual dissolved solids) and gaseous (e.g., CO_2, N_2, N_2O, volatile organics) streams exiting the bioreactor, and most reports only provide partial data on the liquid and biomass fractions. Thus, the objective of this review of recent publications is to gather data on resource recovery, focusing on the possibility of conducting mass balances on carbon and nutrients, in addition to the reported results on the more common performance indicators. Thus, we identify and present the origins of the urban wastewaters used, the reactors, the operation conditions employed, the monitored pollutants, and the results and major conclusions achieved in terms of wastewater remediation, microalgae biomass production and nutrient recovery. An analysis of the mass balance attempts on nutrient fate which have been published is also done, aiming at identifying trends, difficulties and insufficiencies which are limiting the general availability of detailed data.

2. Recent reports on wastewater treatment with microalgae: systems, conditions, and results

Several types of cultivation systems have been and are being studied for the treatment of wastewater with microalgae. In this subchapter, we present the results reported in a poll of 119 scientific papers, published from 2000 to 2021 on wastewater treatment with microalgae, considering different treatment configurations. The rationale for the selection of these publications was to start on recent review papers and, through their references and subsequent citations, enlarge the review basis to original investigation reports, which were subsequently examined. The final selection of studies to comment and quantitative data to show further focused on the objective of the present review, namely, for different scales and reactor types, to compile and analyse the available details on macronutrient recovery or emission mechanisms and their quantification. Systems can be divided in two groups, suspended or fixed cultures. In suspended microalgae cultivation the cells are unattached, to surfaces or each other, in the medium, being this method the most used for wastewater treatment with microalgae (Mohsenpour et al., 2021). To reduce the costs of biomass harvesting processes, and with a focus on wastewater treatment, the implementation of immobilization techniques in microalgae culturing can be considered (Ting et al., 2017).

2.1. Suspended cultures

2.1.1. Laboratory vessels

Many studies have been performed in lab scale conditions using culture vessels that do not relate to any specific type of full-scale system, i.e., resorting to flasks or bottles (e.g., Erlenmeyer flasks, Roux bottles) as reactors. An overview of the results reported for these systems is presented in Table A.1.

First, it is noteworthy that none of the experiments reported here were performed for periods longer than 25 days, a factor that must be taken into consideration when extrapolating the conclusions for application in long term operations. Considering the origin of the wastewater used as a microalgae growth medium, a wide range has been studied, namely the effluents from pre-treatment, primary, secondary, and tertiary treatment stages in conventional wastewater treatment plants (WWTP), as well as the centrate from WWTP sludge digestion. Also, dilution of the effluents or mixtures among

effluents of different origins have been employed. Therefore, a variety of nutrient or pollutant concentrations in the feed media were tested. These variations can affect the removal ability by the microalgae, and the reported studies repeatedly acknowledge this occurrence (Cabanelas et al., 2013b; Kumar et al., 2019; Ling et al., 2019; Lizzul et al., 2014; Ramsundar et al., 2017; Tercero et al., 2014; Wang et al., 2013a; Zhang et al., 2014). Only one study did not present significant differences in the removal yields for the different types of wastewater; however the authors report similar nutrient concentrations in the different wastewaters tested (Abou-Shanab et al., 2014). Thus, the nutrient load and not the specific wastewater origin seems to be a key factor affecting treatment efficiency.

It is also noteworthy that treatments performed to the wastewater before it is fed to the microalgae cultivation experiments can affect the outcome and produce results other than those reported for systems that operate with no pre-treatment. This has been examined for processes used to remove or abate pathogens and other live microorganisms, such as ultraviolet radiation treatment and autoclaving. In fact, some studies have been performed to examine the impact of autoclaving or not the wastewater, and the removal yield values were sometimes different (Lekshmi et al., 2015; Shen et al., 2017; Tran et al., 2021). In these three studies, phosphorus removal was the least affected by the pre-treatment (autoclaving), no pre-treatment being slightly better. While Lekshmi et al. (2015) achieved better nitrate-nitrogen ($N–NO_3$) removal yields when using autoclaved wastewater, Shen et al. (2017) reported the opposite. It was proposed that the presence of nitrifying bacteria in the feed wastewater may have led to the oxidation of ammonium-nitrogen ($N–NH_4$) to $N–NO_3$, leading to lower removal yields for the latter, and hence better results were obtained when the wastewater was autoclaved. In terms of $N–NH_4$ removal, both Lekshmi et al. (2015) and Tran et al. (2021) achieved better results performing no pre-treatment to the wastewater. This may be due to the dominant consumption of this nutrient by other microorganisms as well as microalgae. Therefore, the presence of active microorganisms in the fed wastewater can be beneficial in some situations and prejudicial in others, depending on the microalgae used and the system conditions. It should be noted that the mentioned studies do not provide information on nutrient removal or conversion resulting from the pre-treatments themselves, i.e., before further treatment in microalgae systems.

Some experiments were performed to study alterations in aeration and, depending on the monitored nutrient, the use of carbon dioxide (CO_2)-enriched air can be beneficial or not when compared to ambient air (Kumar et al., 2018; Woertz et al., 2009; Zhou et al., 2012b). The effects of aeration were shown to depend greatly on the system used and the remainder of the operation conditions. If microalgae growth is limited by the carbon content in the wastewater, CO_2 addition can lead to higher growth yields, and consequently higher nitrogen and phosphorus removal. Besides, CO_2 addition can also be advantageous for maintaining ideal pH conditions for microalgae growth, and consequently for attaining higher pollutant removal yields. However, other removal mechanisms taking place in the system will also be affected by CO_2 addition. For instance, if the main nitrogen removal mechanism is ammonia (NH_3) volatilization (stripping), the addition of CO_2, and consequent pH decrease, can impair this mechanism and result in lower nitrogen removal. Also, if CO_2 addition results in lower than ideal pH values in the wastewater, microalgae growth can be affected and lead to lower pollutant removal yields.

Another conclusion that can be taken from this overview is that different removal efficiencies can be achieved by different microalgae in the same conditions. Some authors performed experiments with the same wastewater and operation conditions changing only the inoculated microalgae strain and both the removal and biomass growth yields varied, even with different strains of the same microalga species (Cabanelas et al., 2013a; Fan et al., 2020; Ji et al., 2013; Kshirsagar, 2013; Lekshmi et al., 2015; Li et al., 2011b; Rani et al., 2020; Shen et al., 2017; Sisman-Aydin, 2022; Su et al., 2012b).

2.1.2. Small to full scale open systems

Suspended open systems show great promise for applications of microalgae cultures in wastewater treatment, being easily operated and having low energy requirements (Metcalf & Eddy et al., 2003; Li et al., 2019). An overview of the studies found is presented in Table 1.

Sutherland et al. (2015) performed various experiments in high rate algal mesocosms (HRAM) (cultivated in 15 L plastic buckets), where changes in CO_2 addition were studied through controlling the system at different pH set points. Two 16-day sets of experiments were performed considering no pH control, and maximum pH set points at 8.0, 7.5, 7.0, and 6.5. Both nitrogen and phosphorus removal were best in the experiments with no pH control, its value varying between 8 and 11. However, this may have been due to ammonia volatilization and phosphorus precipitation, rather than microalgae uptake. For the controlled pH experiments nitrogen removal was more efficient at lower pH set points, while phosphorus removal was best when the 7.0 and 7.5 pH set points were used.

Silambarasan et al. (2021) studied a lab scale system (trays) using a consortium of *Chlorella* sp. and *Scenedesmus* sp. for the treatment of a domestic wastewater, achieving high removal yields for the nutrients present, albeit only with diluted wastewater (optimal for a 75% dilution). Frampton et al. (2013) used a similar system, cultivating *Kirchneriella* sp. in 5 L rectangular trays, focusing mainly on the production of lipids for conversion to biodiesel. Xin et al. (2016) tested a multi-layer photobioreactor (PBR) with open trays with a total volume of 1200 L for the continuous treatment of a sludge digestion centrate stream and the production of *Chlorella* sp. Instead of trays, Tran et al. (2021) used plastic tanks to produce *Chlorella variabilis* in the treatment of a domestic effluent. High nitrogen and phosphorus removal efficiencies were attained (>95%), as well as organic carbon removal with a decrease of 83% of Chemical Oxygen Demand (COD). However, due to the operation conditions implemented, ammonia stripping and phosphorus precipitation were significant

Table 1
Published results for microalgae cultivation on wastewater in open systems.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
High rate algal mesocosms (15 L)	*Micractinium bornhemiense* (dominant)	Semi-continuous operation: 16 days (two experiments) HRT: 4 days 3.5–4.1 mmol d^{-1} 22.9–25.8 °C; CO_2 pH: no control | 8 | 7.5 | 7 | 6.5	Primary effluent	DIN: 34.6–56.7 DRP: 4.0–4.7	1st (no control | 8 | 7.5 | 7 | 6.5): DIN: 89 | 52 | 48 | 60 | 64 DRP: 25 | 17 | 13 | 13 | 9 2nd (no control | 8 | 7.5 | 7 | 6.5): DIN: 88 | 54 | 57 | 64 | 66 DRP: 33 | 23 | 28 | 28 | 25	1st (no control | 8 | 7.5 | 7 | 6.5): 168 | 239 | 254 | 261 | 306 mg/L 2nd (no control | 8 | 7.5 | 7 | 6.5): 227 | 270 | 276 | 265 | 293 mg/L	Sutherland et al. (2015)
Plastic tray	*Chlorella* sp. *Scenedesmus* sp.	Operation: 31 days	Domestic (filtered)	$N–NO_3$: 16.58 $N–NH_4$: 37.64 $P–PO_4$: 7.42 TOC: 208.15 COD: 446.25 TN: 61.47	75% | 25%–100% diluted: $N–NO_3$: 96 | 84–96 $N–NH_4$: 98 | 95–98 $P–PO_4$: 95 | 89–95 TOC: 86 | 81–86 COD: 83 | 78–88 TN: 94 | 85–94	75% diluted: 1.35–1.78 g/L General: 0.95–1.78 g/L	Silambarasan et al. (2021)
Rectangular tray (5 L)	*Kirchneriella* sp.	90 μmol photons m^{-2} s^{-1} L:D = 20:4 20 °C; Air+1% CO_2	Municipal (filtered)	TKN: 2.9 TP: 17.0	–	Tray 10%: 0.4* g/L Tray 5%: 0.25* g/L	Frampton et al. (2013)
Plastic tank (50 L)	*Chlorella variabilis* TH03	Operation: 14 days 2100 | 59 620 lx 19.3–23.4 °C Air	Discharge effluent (autoclaved and not)	COD: 124.45–168.16 $N–NH_4$: 65.74–71.9 TP: 4.75–5.73	COD: 83.1 $N–NH_4$: 96.3 TP: 97.1–99.9	1.54 g/L	Tran et al. (2021)
Oxidation pond system	*Chlorella minutissima*	–	Municipal	TN: 95.9 TP: 7 BOD_5: 145	TN: 41 TP: 30 BOD_5: 75	–	Bhatnagar et al. (2010)
Open pond system (20 L)	Mixed culture: cyanobacteria and *Desmodesmus* sp. *Desmodesmus* sp.	Batch Cells residence time: 5 days (desmodesmus) | 19 days (mixed culture)	Urban	TKN: 42.3 $P–PO_4$: 35.4 BOD: 108.3 $N–NH_4$: 29.12 Organic N: 13.2 $N–NO_3$: 23.1 $N–NO_2$: 0.16 Total coliforms: 3×10^7	TN: 55.4–83.9 TP: 30.1–61 Total coliforms: 90–100	Mixed culture: 0.45 g/L 0.017 g L^{-1} d^{-1} Desmodesmus sp: 0.58 g/L 0.013 g L^{-1} d^{-1}	Komolafe et al. (2014)
Raceway (HRAP) (165 L)	*Chlorella pyrenoidosa*	Operation: 19 days 1100 W m^{-2}; L:D = 9:15 6–31 °C; pH: 7.5–9.5	Primary effluent	$N–NH_4$: 46.2 TP: 3.22 COD: 426	$N–NH_4$: 95 TP: 81 COD: 78	1.71 g/L	Dahmani et al. (2016)
Raceway (200 L)	Chlorella sp. *Scenedesmus* sp.	Operation: 10 days 1711 μmol E m^{-2} s^{-1} 9.7 – 24 – 6 °C Air | air+CO_2| air+flue gas	Primary effluent	TN: 22.3–22.7 TP: 9.8–11.6	TN: 94.3–94.9 TP: >97	Chlorella: 0.35–0.5* g/L *Scendedesmus:* 0.35–0.47* g/L	Das et al. (2019)

(*continued on next page*)

factors, as well as nitrification (detected by the increase of nitrate and nitrite concentrations in the outlet). Therefore, not all of the nitrogen and phosphorus removal occurred through microalgae uptake.

Bhatnagar et al. (2010) tested an oxidation pond system for the treatment of municipal wastewater with *Chlorella minutissima* reaching removal efficiencies for nitrogen and phosphorus, 41 and 30%, respectively, lower than for Biochemical Oxygen Demand (BOD_5) removal (75%). Komolafe et al. (2014) tested an open pond system for the treatment of

Table 1 (*continued*).

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Raceway (HRAP) (500 L)	*Chlorella variabilis* TH03	Semi-continuous operation: 148 days HRT: 14–21 days 102–815 μmol m^{-2} s^{-1} 17–35 °C; pH: 8.3–9.5	Domestic	COD: 59.8–193.9 N–NH_4: 14.8–58.9 TN: 16.3–61.7 P–PO_4: 0.61–15.5 TP: 0.61–15.5 N–NOx: 1.5–7.2	COD: 64.7–90.7 N–NH_4: 88.9–98.0 TN: 85.1–96.8 P–PO_4: 99.7–100 TP: 99.7–100	1.67–1.85 g/L 11.1–15.3 g m^{-2} day^{-1}	Do et al. (2020)
Raceway (HRAP) (500 L)	*Chlorella variabilis* TH03	Batch operation: 17 days (no CO_2) 15 days (CO_2) 2100 | 59 620 lx 21.1–27.2 °C CO_2| Not aerated pH: 8.9–9.8	Discharge effluent (autoclaved and not)	COD: 124.45–168.16 N–NH_4: 65.74–71.9 TP: 4.75–5.73	COD: 89.8 N–NH_4: 97.7 TP: 97.1–99.9	No CO_2: 13.1 g m^{-2} d^{-1} 1.72 g/L CO_2: 38.5 g m^{-2} d^{-1} 3.85 g/L	Tran et al. (2021)
Raceway (HRAP) (530 L)	*Scenedesmus obliquus*	Batch (B) and Continuous (C) operation: 53 days (B), reaching 157 days (C) HRT: 7,8,9,10 days (B), 10 days (C); pH: 8–9.5	Secondary effluent	TN: 24.92–26.16 TP: 1.77–2.23	TN: 65.12 TP: 58.78	8.26 g m^{-2} d^{-1}	Arbib et al. (2013)
Raceway (HRAP) (470 L)	*Chlorella* sp. was dominant	Operation: 1 year HRT: 4 | 6 | 8 days 234–446 W m^{-2} 13.1–23.7 °C	Primary effluent	N–NH_4: 26–36 COD: 318–463	N–NH_4: 95–99 COD: 56–94	3.3–25.8 g m^{-2} d^{-1} 11.3–20.9 g/L	Gutiérrez et al. (2016)
Raceway (HRAP) (462 L) A | B	–	Continuous/Semi-continuous operation: 1 year HRT: 7–10 (A) | 4–8 days (B); 13–20 °C	Primary effluent	TN: 51 TP: 8.5	TN: 73 (A) | 57 (B) TP: 43 (A) | 32 (B)	150–410 g TSS m^{-3}	García et al. (2006)
Raceway (HRAP) (470 L)	Mainly composed of *Chlorella* sp.	Continuous operation: 3 months HRT: 8 days (50 days) and 4 days (40 days)	Primary effluent	–	HRT (4 | 8): TSS: 80 (4) | 94 (8) CODtot: 64 (4) | 74 (8) TC: 60 (4) | 73 (8) TP: 70 (4) | 84 (8) TN: 53 (4) | 76 (8) N–NH4: 91 (4) | 94 (8) Total Se: 46 (4) | 43 (8)	0.42 g DW/L	Li et al. (2021)
Raceway (HRAP) (4 × 9.6 m^3)	Inoculum mainly composed by the genera *Coelastrum*	Continuous operation: 1 year HRT: short (S) (3,5,7) | long (L) (5,7,10) days CO_2 (C) | Not aerated (NC); pH: 7–9.5	Anaerobic digester effluent	N–NH_4: 46.1–58.1 P–PO_4: 7.7–8.6 COD: 140–178 TOC: 61.2–74 TKN: 49.2–64.5 TP: 8.11–9.2	S NC | S C | L NC | L C: TN: 53 | 51 | 62 | 53 P–PO_4: 47.4–61.6 | 52.3–55.8 | 52.6–61.9 | 54.7–59.0 N–NH_4: 74.9–81.7 | 63.2–85.0 | 84.7–93.6 | 76.9–94.1	S: 8.4–29.3 g m^{-2} d^{-1} L: 6.5–19.2 g m^{-2} d^{-1}	De Godos et al. (2016)

(*continued on next page*)

a wastewater with a lower nitrogen/phosphorus ratio (around 2 instead of around 14) than that reported by Bhatnagar et al. (2010) achieving higher removal yields for both nutrients.

The raceway systems are the most used open systems worldwide, so many studies have been carried out in the wastewater treatment sector employing these systems at long operation periods. The studies found used mainly the *Chlorella* and *Scenedesmus* genera as inocula and focused on the treatment of primary effluents.

Table 1 (*continued*).

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Raceway (HRAP) (700 L (A) | 800 L (B) | 850 L (C))	*Scenedesmus* sp.	Semi-continuous 300–468 W m^{-2} 13–23 °C Stage I: Operation: 29 days HRT: 2.7 days pH: 9 (A) | 8 (B) | 7 (C) CO_2 Stage II: Operation: 18 days HRT: 2.8 days pH: 9 (A) | 8 (B) | 7 (C) Flue gas Stage III: Operation: 32 days HRT: 6.7 days pH: 8; Flue gas Stage IV: Operation: 18 days HRT: 6 days	Primary effluent	COD: 432–744 TOC: 181–313 IC: 117–140 TN: 52–75 $N–NH_4^+$: 50–74 $N–NO_3^-$: 0–1 $P–PO_4^{-3}$: 9–11	Stage I: COD: 88 (A) | 88 (B) | 81 (C) TOC: 71 (A) | 73 (B) | 68 (C) TN: 69 (A) | 73 (B) | 65 (C) TP: 41 (A) | 40 (B) | 34 (C) Stage II: COD: 91 (A) | 88 (B) | 92 (C) TOC: 85 (A) | 83 (B) | 83 (C) TN: 60 (A) | 75 (B) | 62 (C) TP: 61 (A) | 63 (B) | 65 (C) Stage III: COD: 86 (A) | 87 (B) | 88 (C) TOC: 72 (A) | 74 (B) | 75 (C) TN: 83 (A) | 93 (B) | 81 (C) TP: 64 (A) | 68 (B) | 71 (C) Stage IV: COD: 73 (A) | 79 (B) | 68 (C) TOC: 60 (A) | 56 (B) | 58 (C) TN: 97 (A) | 98 (B) | 97 (C) TP: 62 (A) | 61 (B) | 56 (C)	4–17 g m^{-2} d^{-1}	Posadas et al. (2015)
Raceway (HRAP) (4 × 4375 m^3)	*Micractinium pusillum* (dominated)	operation: 1 year HRT: 9 (winter) | 7 (autumn, spring) | 5.5 (summer) days 1200 (winter) | 3900 (summer) μmol d^{-1} 7.2–17.7 °C	Primary effluent	$N–NH_4$: 20.0–30.7 DRP: 0.9–3.6	Autumn | Winter | Spring | Summer: $N–NH_4$: 47 | 53 | 79 | 77 DRP: 37 | 22 | 49 | 20	Annual average algal biomass of 6.6 g m^{-2} d^{-1}	Sutherland et al. (2014)

Dahmani et al. (2016) used a 165 L high-rate algal pond (HRAP) accomplishing removal yields of 81% for total phosphorus (TP) and 95% for ammonia nitrogen, their main removal mechanism being algae assimilation, and a 78% for COD. Do et al. (2020) used an HRAP system with a larger volume (500 L) operating in semi-continuous regimen and achieved almost complete phosphorus removal (>99.7%), as well as high levels of ammonia nitrogen (88.9 to 98.0%) and total nitrogen (85.1 to 96.8%) removal. COD removal yields between 94.7 and 90.7% were also accomplished. Tran et al. (2021) also achieved high removal yields for TP (>97%), ammonia nitrogen (97.7%) and COD (89.8%) using the same system as Do et al. (2020) operated in batch mode. Arbib et al. (2013) operated a 530 L HRAP in batch and continuous modes for 157 days, however accomplishing lower removal yields than in the two previous studies, namely, 58.78% for TP. Since there was no pH control, the culture reached values of pH higher than 9, which indicates that the nitrogen removal occurred also through ammonia stripping and not only by algae uptake.

Das et al. (2019) also used an HRAP system to study the influence of supplementation with CO_2 or flue gas on the production of *Chlorella* and *Scenedesmus* biomass and on wastewater treatment performance. A higher biomass production was achieved with CO_2 or flue gas addition for both genera. Also, supplementation was intermittent allowing pH control, unlike the system with no gas injection. Thus, although the reduction in nutrient concentrations in the wastewater was similar, the removal mechanisms involved could have been different.

Gutiérrez et al. (2016) operated a 470 L HRAP for 1 year changing the hydraulic residence time (HRT) according to the season, accomplishing removal yields between 56% and 94% for chemical oxygen demand (COD) and higher than 95% for ammonia nitrogen, depending on the weather conditions. García et al. (2006) also performed a yearlong operation of two

HRAPs with the specific objective of studying the changes in pollutant removal efficiencies caused by changes in HRT. One of the reactors was always operated with higher HRT values than the other and both were changed according to the season. The reactor operated with higher HRT values achieved higher total phosphorus and total nitrogen removal yields (43% and 73%, respectively). In this study, the main removal mechanisms for phosphorus and nitrogen were chemical precipitation and ammonia volatilization, respectively (no pH control and culture reached pH values above 9). Li et al. (2021) performed a continuous operation experiment for 3 months in a 470 L HRAP also testing different HRT settings. Similarly, higher removal yields were achieved for higher HRT and, although part of the nutrients was assimilated by the microalgae, the precipitation and volatilization mechanisms could not be overlooked. De Godos et al. (2016) tested shorter and longer HRT values in a yearlong operation of four raceways, also achieving higher nitrogen removal with longer HRT. Mass balances were performed to determine the nutrient removal mechanisms taking place in the system. Microalgae uptake removed between 17 and 57% of the total nitrogen present in the inlet, depending on the season and operation conditions, while the volatilized nitrogen ranged from 2 to 47% and the oxidized (nitrified) nitrogen from 5 to 32%.

Posadas et al. (2015) operated three HRAP raceways with different volumes, namely 700, 800 and 850 L, for 97 days, three studies being performed during this time. In the first stage, the influence of pH was studied through the addition of pure CO_2 to control the maximum pH value at a different set point for each HRAP. In this stage, there were no significant differences between the pollutant removal performances of the raceways with different pH set points. In the second stage, the influence of the source of CO_2 was studied by using flue gas instead of pure CO_2. In comparison to the first stage, the removal yields were slightly higher for COD, total organic carbon (TOC) and TN and significantly higher for TP. Thus, the microalgae adapted well to the use of flue gas instead of pure CO_2, achieving better results. In the last two stages, the influence of the presence or absence of CO_2 supply was studied, by using flue gas in stage III with a higher HRT than the previous stage, and no CO_2 addition in stage IV. The conditions in stage III allowed higher removal yields of COD, TOC, and TP but the removal of TN was higher in stage IV. During both the latter stages the main mechanism for nitrogen removal was ammonia stripping, while for phosphorus removal it was biomass uptake. In conclusion, although the injection of flue gas allowed pH control, it did not significantly enhance wastewater remediation in terms of nitrogen.

Sutherland et al. (2014) performed a yearlong experiment with four 4375 m^3 raceways in order to study the operation under all the season-related variations and to identify the complications arising from full-scale treatment. During spring and summer, nitrogen removal was enhanced due to the more favourable temperatures, photoperiod and irradiation conditions for microalgae growth, and consequently for a higher pollutant uptake by these. On the other hand, phosphorus removal was greater during autumn and spring. The changes in removal yields were also suggested to be related to the changes in the influent composition depending on the season.

2.1.3. Small to full scale closed systems

Typically closed systems allow better control over the culture environmental parameters and have a higher surface area to volume ratio when compared to conventional open systems, generally improving light absorption. However, these systems involve higher investment and operational costs, and each type presents specific limitations associated to their design (Mohsenpour et al., 2021).

2.1.3.1 Tubular systems Tubular systems with many different designs have been proposed, a selection of studies using them being presented in Table 2.

di Termini et al. (2011) studied the changes occurring in indoor and outdoor experiments using two lab scale horizontal tubular photobioreactors (PBR) to treat a secondary effluent from a wastewater treatment plant. In this study, the inoculum was obtained through spontaneous growth on the same wastewater, resulting in a consortium dominated by the *Scenedesmus* genus, which was used instead of a pure culture of a specific microalga. While in the indoor experiment continuous illumination was applied throughout the 7-day operation, the outdoor experiment was run under a daily light–dark cycle. Although the maximum irradiance was higher than in the laboratory conditions during the light period, this was apparently insufficient to compensate for the dark period and thus a higher biomass growth yield was obtained in the indoor experiment. Also, higher pollutant removal yield values were achieved in the indoor experiment, which confirmed the favourable effect of the better control of the operational conditions, leading to better results. However, there are limitations to the indoor operation of large-scale systems, and although the outdoor N and P removal yields were lower than those obtained indoors, 90% levels could be reached, which can be sufficient to produce an effluent within regulatory discharge limits. In terms of the removal mechanisms, the authors concluded that microalgae uptake was the primary removal mechanism.

To determine the ideal operation conditions for the cultivation of *Chlorella protothecoides* on a secondary effluent previously filtered to remove endogenous microorganisms, Binnal and Babu (2017) tested different light and dark cycles and irradiance levels in a lab-scale horizontal tubular PBR. They achieved distinct results in each condition, concluding that the cultivation yields, and water treatment quality was highly affected by these parameters. The optimal operation condition they identified allowed complete removal of nitrogen and phosphorus and almost 80% COD removal. However, it is noteworthy that irradiance conditions typically cannot be controlled in outdoor operations and vary with the seasons. In terms of phosphorus recovery, at higher pH the main removal mechanism was precipitation while at lower pH it was microalgae uptake.

Table 2
Published results for microalgae cultivation on wastewater in tubular systems.

Reactor	Inoculum	operation conditions	Wastewate type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Horizontal tubular PBR (8 L | 18 L)	Spontaneous blooming from wastewater mainly composed of genus *Scenedesmus*	Batch operation: 7 days Indoor: 200 μE m^{-2} s^{-1} L:D = 24:0; 20 °C Outdoor: 1300 μE m^{-2} s^{-1} 4–28 °C	Secondary effluent	N–NH_4: 7.43–16.23 P–PO_4: 0.99–2.14	Indoor: N–NH_4: 99.9 P–PO_4: 99.9 Outdoor: N–NH_4: 90 P–PO_4: 80–90	Indoor: 0.25 g L^{-1} d^{-1} 5.81 g/d Outdoor: 0.16 g L^{-1} d^{-1}	di Termini et al. (2011)
Horizontal tubular PBR (5 L)	*Chlorella protothecoides*	Operation: 10 days 6 klux; L:D = 16:8 25 °C; Air+6% CO_2	Secondary effluent (filtered)	COD: 48.25 TN: 14.56 TP: 2.25	COD: 78.03 TN: 100 TP: 100	1.96 g/L	Binnal and Babu (2017)
Biocoil (15 L)	*Chlamydomonas reinhardtii*	Operation: 10 days 220 μmol photons m^{-2} s^{-1} L:D = 24:0; 25 °C	Centrate	TKN: 128.6 TP: 120.6	TN: 83 TP: 14.45	2.0 g L^{-1} d^{-1}	Kong et al. (2010)
Horizontal tubular PBR (1200 L)	Lake water (microalgae and bacteria consortia)	Continuous operation: 1 month (pI) | 2 months (pII) HRT: 8 (pI) 12 (pII) days 5–25 °C; Air	Toilet wastewater	COD: 398–462 N–NH_4: 79–121 TP: 7–15	pI | pII: COD: 84 | 60 N–NH_4: 86 | 98 TP: 50 | 89	pI: 303 mg/L pII: 265 mg/L	Hom-Diaz et al. (2017)
Tubular PBR (31 L)	Mixed microalgal–bacterial consortium obtained from an HRAP treating domestic wastewater	Operation: 40 HRT: 10 days 74 μmol m^{-2} s^{-1} L:D = 16:8; 20–25 °C	Primary effluent	TOC: 167 IC: 122 TN: 106 N–NH_4: 86 P–PO_4: 12	TOC: 85 IC: 78 TN: 80 N–NH_4: 100 P–PO_4: 68	–	Posadas et al. (2014)
Airlift tubular PBR (380 L)	*Scenedesmus obliquus*	Batch (B) Continuous (C) operation: 45 days (B), reaching 157 days (C) HRT: 2,3,4,5 days (B), 5 days (C); Air; pH: 8–9.5	Secondary effluent	TN: 24.92–26.16 TP: 1.77–2.23	TN: 89.68 TP: 86.71	21.76 g m^{-2} d^{-1}	Arbib et al. (2013)

Kong et al. (2010) performed a one-month experiment with a 15 L biocoil system in order to produce a specific microalga on a sludge digestion centrate feed. The objective of this work was to determine the adequacy of this feed to produce this strain and the accumulation extent of lipids in the microalga which could be used to produce biodiesel, as well as the nutrient removal performance of the system in the tested conditions. Although a high nitrogen removal was achieved, phosphorus removal was low, probably due to a low N:P ratio in the centrate. The authors proposed that nutrient removal was due to microalgae uptake in the conditions used. However, good biomass productivity (2.0 g L^{-1} day^{-1}) was achieved with a lipid content of 25% on a dry basis (average values between 1 and 40% have been reported by Richmond, 2004), so in terms of biodiesel production the process showed potential. However, when considering the wastewater treatment performance, it was not efficient and would not be feasible unless the resulting stream rich in phosphorus could be used in other applications.

Hom-Diaz et al. (2017) performed experiments with toilet wastewater using a pilot scale horizontal tubular PBR inoculated with water from a natural lake. Two HRT values were tested, COD removal being more efficient with lower HRT, while the nitrogen and phosphorus removal yields were higher for higher HRT. In this work, the removal of pharmaceutical compounds was also studied. In both experiments, the microalgae culture presented consistently high removal rates for many of the pharmaceutical compounds tested, namely acetaminophen (>99%), ibuprofen (>98%), atenolol (>85%), paroxetine (>93%), furosemide (∼100%), erythromycin (>84%), alprazolam (>87%), and azithromycin (>88%), even though they were already present in small concentrations in the influent. These results show that wastewater treatment with microalgae systems can provide the additional advantage of removing relevant pharmaceutical compounds. Hom-Diaz et al. (2017) report only on the overall removal levels of these compounds, therefore studies to determine the removal mechanisms are still lacking and would help to validate this technology as a remediation option for pharmaceutical compounds.

Posadas et al. (2014) used a microalgal–bacterial consortium from another reactor as inoculum in order to study the treatment of a primary effluent in a small scale (31 L) tubular PBR. A 16:8 h light:dark cycle was established, continuous

Table 3
Published results for microalgae cultivation on wastewater in flat systems.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Flat-shaped glass flask (500 mL)	*Tetraselmis* sp. (Ts), *P. kessleri* (Pk) and *C. saccharophilum* (Cs)	Batch operation: 9–12 days 130 μmol m^{-2} s^{-1}; L:D = 24:0 25 °C; Air+2% CO_2	Activated sludge dehydration effluent (autoclaved A | not NA)	N–NH_3: 22–100 P–PO_4: 49–65	Ts A | Ts NA | Pk A | Pk NA | Cs NA: N–NH_3: 98 | 99 | 98 | 99 | 99 P–PO_4: 82 | 75 | 20 | 25 | 39	Ts A | Ts NA | Pk A | Pk NA | Cs NA: 157 | 133 | 101 | 125 | 127 mg L^{-1} d^{-1}	Aketo et al. (2020)
Flat panel airlift PBR (10 L)	*Chlorella kessleri* (Ck), *Chlorella vulgaris* (Cv) and *Nannochloropsis oculate* (No)	Batch operation: 11 days 100 μmol m^{-2} s^{-1}; L:D = 24:0 25 °C; Air+CO_2	Centrate (diluted with pre-treatment effluent)	Centrate: TN: 1233 TP: 11.90 Pre-treatment ef.: TN: 39–65 TP: 3.1–5.4	TN: 96 (Ck) | 95 (Cv) | 47 (No) TP: 99 (Ck) | 98 (Cv) | 96 (No)	*Ck*: 2.70 g/L *Cv*: 2.91 g/L *No*: 1.05 g/L	Caporgno et al. (2015)
Flat panel PBR (4.5 L)	*Scenedesmus obliquus*	Batch | continuous HRT: 0.5 | 1.1 | 1.7 | 2.3 | 2.8 | 3.4 days 250 μmol m^{-2} s^{-1}; L:D = 14:10 20 °C; Air+5% CO_2	Secondary effluent	TN: 15.2–34.9 TP: 0.81–3.56	HRT = 0.5 | 1.1 | 1.7 | 2.3 | 2.8 | 3.4 days: TN: 0 | 90 | 91 | 89 | 87 | 81 TP: 0 | 95 | 97 | 90 | 98 | 95	HRT = 0.5 | 1.1 | 1.7 | 2.3 | 2.8 | 3.4 days: 0 | 0.35 | 0.36 | 0.28 | 0.38 | 0.29 g L^{-1} d^{-1}	Ruiz et al. (2013)
Airlift panel PBR (30 L)	*Scenedesmus obliquus*	Semi-continuous operation: 20 days HRT: 6 days (I) | bio-mass dependent (II); <30 °C; Air	Primary effluent	N–NH_4: 12.72–40.55 P–PO_4: 1.12–2.69	N–NH_4: 83.63 (I) | 76.57 (II) P–PO_4: 84.78 (I) | 70.68 (II)	(I): 0.07 g L^{-1} d^{-1} (II): 0.22 g L^{-1} d^{-1}	Ling et al. (2019)
Flat panel PBR (3 × 50 L)	*Chlorella sorokiniana*	Batch operation: 4 weeks 196 μmol m^{-2} s^{-1}; L:D = 16:8 30 °C; Air	Anaerobic reactor effluent	N–NH_3: ∼200–300 P–PO_4: ∼15.20	N–NH_3: 100 P–PO_4: 40–60	90–130 mg L^{-1} d^{-1}	Leite et al. (2019)
Flat panel PBR	*Chlorella sorokiniana*	Continuous 400 μmol m^{-2} s^{-1} 37 °C; Air+CO_2	Mixed influent indus-trial/municipal	COD: 386.9 TN: 48.6 TP: 7.2 N–NH_4: 46.7	Dilution rate = 4.32 | 3.6 | 1.8 | 0.72 d^{-1}: COD: 53.9 | 52.3 | 47.8 | 53.6 TN: 34.4 | 41.9 | 67.6 | 94.2 TP: 45.7 | 55.9 | 73 | 82.7 N–NH_4: 19.9 | 13.4 | 38.9 | 96.9	0.18 (4.32) | 1.44 (0.72) g/L 0.8 (4.32) | 1.46 (1.8) | 0.95 (0.72) g L^{-1} d^{-1}	de Francisci et al. (2018)

illumination being provided by light bulbs. Five stages were considered, changing the HRT value. The best removal efficiencies were achieved in stage I with the HRT set at 10 days, when the lowest removal yield was measured for phosphorus (68%), while the other pollutant parameters showed higher removal values (>78%). By performing mass balances, it was concluded that the main nitrogen and phosphorus removal mechanism was the uptake by microalgae and bacteria, since most of the removed nitrogen and phosphorus was quantified in the recovered biomass.

Arbib et al. (2013) performed a long-term operation with a pilot scale airlift tubular PBR to treat the effluent from the secondary stage of a WWTP. Good average removal efficiencies were achieved, and the European regulatory discharge requirements were met in the complete operation period. In this study, the reactor was inoculated with a specific strain, but the microalgae community was not subsequently monitored, so no guarantee was provided that the biomass produced was from the inoculated strain. Also, it is noteworthy that the reactor was operated under outdoor conditions, and the results can therefore be directly compared with those reported for other outdoor systems. In terms of removal mechanisms, the authors considered that in the established conditions phosphorus removal was mainly due to microalgae uptake.

2.1.3.2 Flat panel systems Many studies have been performed using different flat panel systems for the treatment of domestic wastewater with microalgae cultures, and Table 3 presents a selection of them.

Aketo et al. (2020) performed laboratory scale experiments with flat-shaped glass flasks in order to determine the nitrogen and phosphorus removal abilities of three different microalgae species. For these experiments the effluent from an activated sludge dewatering process was used (previously autoclaved or not). Considering nitrogen removal, all microalgae achieved almost complete removal, however *Tetraselmis* sp. allowed the best phosphorus removal, while the other two showed poor phosphorus uptake abilities (under 40%). Comparing the results obtained with the autoclaved and not autoclaved wastewater, the nutrient removal performances were essentially the same (maximum difference of 5%).

Caporgno et al. (2015) also performed experiments with different microalgae in a laboratory scale (10 L) flat panel airlift PBR for the treatment of sludge digestion centrate. However, a dilution of the latter with pre-treated wastewater was performed to bring nutrient concentrations down to levels acceptable for the microalgae culture. The three microalgae tested showed similar phosphorus removal abilities (almost 100%). Nonetheless, *Nannochloropsis oculate* achieved much lower removal yield (47%) in terms of nitrogen abatement than the other two species (almost 100%).

Ruiz et al. (2013) cultivated *Scenedesmus obliquus* in secondary effluent with different HRT values (0.5 to 3.4 days) in a flat panel PBR. The results achieved were similar for the HRT range above 1.1 days (above 87% for both nitrogen and phosphorus), but a HRT of 0.5 days resulted in no removal of nitrogen or phosphorus due to the washout of the microalgae from the system. Ling et al. (2019) also performed experiments with *Scenedesmus obliquus*, this time cultivated on the effluent from primary wastewater treatment in a 30 L airlift flat panel PBR. Two different operation conditions were tested (both outdoors), the first with the HRT set at 6 days and the second at fixed biomass concentration. Between the two, the control of HRT at 6 days allowed a higher removal efficiency for both nitrogen and phosphorus.

The treatment of an anaerobic reactor effluent in three 50 L flat panel PBRs inoculated with *Chlorella sorokiniana* was studied by Leite et al. (2019). Complete nitrogen removal was achieved while phosphorus removal varied between 60 and 100%. However, ammonia stripping was the main mechanism for nitrogen removal and not microalgae uptake. de Francisci et al. (2018) also cultivated *Chlorella sorokiniana* in a flat panel PBR, this time on mixed industrial and municipal wastewater at different dilutions rates. In terms of COD removal, the different dilution rates lead to similar removal values (47.8 to 53.9%), however in terms of TN, TP, and N–NH_4^+, removal yields were higher when lower rates (i.e., higher HRT) were applied.

2.1.3.3 Cylinder PBR systems Table 4 presents an overview of reports on cylinder type PBR systems used for microalgae cultivation in urban wastewater.

Sydney et al. (2011) performed tests using a BioFlo reactor with different microalgae strains to determine which would grow best on secondary wastewater treatment effluent, removing the most nitrogen and phosphorus while accumulating the highest lipid content. *Botryococcus braunii* presented the best results in the conditions tested, with complete phosphorus removal, substantial nitrogen removal (79.63%) and high lipid content in the biomass (36.14% on a dry basis).

Ruiz-Martinez et al. (2012) tested, using a cylinder bubble column reactor, the remediation of an anaerobic membrane bioreactor effluent with a mixed microalgae culture for which the inoculum was collected from the secondary clarifier of the same WWTP. High phosphorus and nitrogen removal yield were obtained, and because the pH of the culture was kept at 7.2, these results were considered to be due to microalgae uptake rather than volatilization and precipitation phenomena.

The use of a mixed culture of microalgae and activated sludge for the treatment of primary wastewater treatment effluent was tested by Su et al. (2012a). In this work, different algae:sludge concentration ratios were tested, from 10:1 to 1:5, and both the microalgae and sludge alone were tested as well. The best nitrogen and phosphorus removals, 91 and 91.4%, respectively, were achieved with an algae:sludge ratio of 5:1, much higher than both the microalgae and sludge individual results. In these conditions, the mass balances indicated that 60.0 and 91.4% of the nitrogen and phosphorus removed, respectively, were recovered in the biomass, only 4.6% of the nitrogen being removed through nitrification. Therefore, this process allowed the recovery of most of the nutrient load from the wastewater, to be used within a circular economy.

Cho et al. (2013) tested four types of wastewaters from different steps in a WWTP, as feed for microalgae cultivation, to determine the one that allowed higher biomass yields and higher lipid content in the latter. In terms of water remediation, almost complete nitrogen and phosphorus removal was obtained with the primary effluent, the mixture of primary effluent with anaerobic digestate, and the digestate mixed with wastewater rejected from the sludge thickening and dewatering processes. The sludge thickening and dewatering wastewater was the only tested feed that led to notably lower nutrient removal efficiency. The wastewater mixtures allowed the best biomass growth yields, and that using the thickening and dewatering effluents produced the highest biomass concentration (3.01 g/L). The use of unmixed effluent from the primary stage led to a 41.5% lipid content in the microalgae biomass, much higher than any of the other feeds tested.

A mixture of wastewater treatment effluents was also studied by Arias et al. (2018) for microalgae cultivation, namely effluents from the secondary treatment and sludge anaerobic digestion, at a volume ratio of 50:1, respectively. In this case, the sludge digester received both the sludge from the main treatment and the harvested biomass from the microalgae cultivation. For the latter a cylinder bioreactor was used, inoculated with a sample from an existing HRAP treating

Table 4
Published results for microalgae cultivation on wastewater in cylinder systems.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
BioFlo reactor (11 L)	*Chlorella vulgaris* (Cv) *Botryococcus braunii* (Bb)	Operation: 14 days 3500 lux; L:D = 12:12 25 °C; Air+5% CO_2	Secondary ef.	N–NO_3: 0.2 P–PO_4: 2.0	Cv | Bb: N–NO_3: 73.77 | 79.63 P–PO_4: 100 | 100	1.01 (Cv) | 1.88 (Bb) g/L	Sydney et al. (2011)
Cylinder bubble column (10 L)	Sample from the walls of the secondary clarifier in the WWTP	Semi-continuous operation: 42 days HRT: 2 days | SRT: 2 days 143–209 μE m^{-2} s^{-1}; L:D = 24:0; 28–32 °C; CO_2	Submerged anaerobic membrane bioreactor ef.	N: 40–80 P: 5–11	N: 67.2 P: 97.8	Maximum: 595 mg/L Average: 467 mg/L	Ruiz-Martinez et al. (2012)
Stirred tank PBR (14 L)	Sample from the secondary clarifier (mainly filamentous blue-green algae) and activated sludge	Operation: 14 days 7000 lux; L:D = 12:12; 13 °C	Primary ef.	TN: 50.1 P–PO_4: 8.8 COD: 380 N–NH_4^+: 39.4 N–NO_3^-: 0.02 N–NO_2^-: 0 TKN: 50.08	A:S (1:0 | 10:1 | 5:1 | 1:1 | 1:5 | 0:1): TN: 41.7 | 58.6 | 91.0 | 86.0 | 50.2 | 18.6 P–PO_4: 54.4 | 64.0 | 93.5 | 82.9 | 72.7 | 10.6 COD: 66.0 | 91.2 | 95.8 | 96.2 | 94.0 | 73.6 TKN: 89 | 93.7 | 95.8 | 93.7 | 93.7 | 31.4 N–NH_4^+: 95 | 100 | 100 | 100 | 100 | 23	–	Su et al. (2012a)
Cylindrical PBR (1 L)	*Chlorella* sp. ADE5 isolated from the anaerobic digester	Operation: 5 days 200 μmol m^{-2} s^{-1}; L:D = 24:0 30 °C; Air+1% CO_2	Primary ef. (PS), Anaerobic digester ef. (AD), Conflux of wastewaters from sludge-concentrate tanks and dewatering facilities (CR)	PS | CR | AD+PS | AD+CR: TN: 50*| 125*| 160*| 250* TP: 7*| 15* | 11*| 17*	PS | CR | AD+PS | AD+CR: TN: 100 | 70 | 100 | 98 TP: 100 | 67 | 100 | 100	PS | CR | AD+PS | AD+CR: 1.58* | 1* | 1.25* | 3.01* g/L	Cho et al. (2013)
Cylindrical PBR (30 L)	HRAP sample (*Chlorella* sp., *Scenedesmus* sp. and *Stigeoclonium* sp.)	Continuous operation: 30 days HRT: 8 days 289 μmol m^{-2} s^{-1}; L:D = 12:12 25–29 °C; Air	Digestate diluted in secondary effluent	N–NO_3: 15.94 sCOD: 141.1 N–NH_4: 9.17 P–PO_4: 2.18	N–NO_3: 58 COD: 70 N–NH_4: 100 P–PO_4: 100	1.1 gTSS/L	Arias et al. (2018)
Bubble column system (3.2 L)	*Chlorella vulgaris*	Batch operation: 12 days 177 μmol m^{-2} s^{-1}; L:D = 18:6; 20 °C; Air	Primary effluent (autoclaved; filtered F/not NF)	N–NH_4: 104.51 P–PO_4: 23.65	F | NF: N–NH_4: 94.18 | 95.22 P–PO_4: 97.69 | 96.63	F | NF: 0.51 | 0.53 g/L 0.164 g L^{-1} d^{-1} (av.)	Mayhead et al. (2018)

municipal wastewater. Complete phosphate and ammonia nitrogen removal was achieved in this cultivation and a steady biomass production was obtained (1.1 g/L), with a relevant contribution to the overall biogas production in the WWTP.

Mayhead et al. (2018) performed experiments to determine the impact caused by previous filtration of the wastewater used in a microalgae cultivation system in a bubble column reactor. The wastewater used was an autoclaved primary treatment effluent, and high phosphate and ammonia nitrogen removal yields were obtained in both filtered and unfiltered wastewater experiments (above 90%). Considering the operation conditions tested, the authors concluded that the principal removal mechanism for both ammonia nitrogen and phosphorus was microalgae uptake.

2.2 Fixed biomass systems

Microalgae immobilization methods are generally divided in two categories: Self-attachment and entrapment (Mohsenpour et al., 2021). In addition to facilitating the harvesting process, it has been observed that the high concentration of active biomass within biofilms or other matrices leads to an increased rate of biodegradation, resulting in improved pollutant removal efficiencies. This effect could also be attributed to the fact that particulate, organic and inorganic compounds can accumulate at the surface of the immobilizing polymers or biofilms, increasing and sustaining a high concentration of these substances in the proximity of the microalgae and other active microorganisms, in effect facilitating the biodegradation process (Mohsenpour et al., 2021).

Also, the immobilization of co-cultures of microalgae and bacteria can be beneficial, since this provides the generation of O_2 and CO_2 in close proximity, avoiding diffusional limitations within the culture medium or immobilizing matrix (Mohsenpour et al., 2021).

2.2.1 Self-attachment

Microalgae can attach to the solid, rough surface of a supporting material and form a biofilm. In most reported cases, the biofilm is actually a bacteria–microalgae consortium (Li et al., 2019; Ting et al., 2017). Table 5 presents a selection of studies performed using technologies taking advantage of this self-attachment ability.

Boelee et al. performed two works (Boelee et al., 2011, 2014) using a flow cell system that consists of a biofilm reactor where the microalgae are inoculated onto a plastic sheet and the wastewater flows across the surface of the formed biofilm. The inoculum used for both works was scraped off the surface of a settling tank in the WWTP providing the feed medium and where the biofilm system was assembled. It is noteworthy that, although the two inocula were taken from the same settler, different microalgae were identified in the subsequently formed biofilm, confirming that the same operational conditions can support different microalgae species. These studies showed that microalgae/bacterial biofilms can efficiently assimilate nitrogen and phosphorus, being a possible option for the tertiary treatment in wastewater treatment plants.

Sukačová et al. (2015) performed batch and continuous experiments in a horizontal flat panel PBR with synthetic wastewater and the effluent from secondary wastewater treatment. In the batch experiments a 12:12 light and dark cycle was used, and phosphorus removal was directly dependent on the light intensity, being lower for low irradiance levels, as expected. In the continuous experiments both continual illumination and the 12:12 cycle were tested, and phosphorus removal efficiency was much higher under continuous irradiation. This led to the conclusion that the best option would be to use solar irradiance during the day and artificial light during the night in order to maintain high phosphorus removal efficiency and still save energy during the day. In this system, the impact of precipitation and dissolution of phosphorus caused by the pH changes was found to be important.

Laboratory scale experiments were performed with domestic grey water and anaerobically digested slurry to determine the COD, nitrogen, and phosphorus removal efficiencies in a biofilm reactor (Choudhary et al., 2017). For these experiments a microalgae consortium obtained from wastewater treatment ponds was used so that it was adapted to the pollutants present in the feed effluents. Overall, good nitrogen and phosphorus removal yields were achieved (above 88%) as well as sufficient (according to European regulations) COD removal. The authors claimed that the system used is scalable and employs affordable resources, being an effective and economically viable option for wastewater treatment.

Posadas et al. studied the use of a biofilm open PBR for the treatment of primary (Posadas et al., 2013) and secondary (Posadas et al., 2014) effluents using microalgae/bacteria consortia. In both works high carbon, nitrogen and phosphorus removal yields were achieved, confirming the advantages of using mixed bacterial and microalgae cultures. The main carbon and phosphorus removal mechanism was biomass assimilation while ammonia stripping was a significant mechanism for nitrogen removal.

Shi et al. (2014) tested a prototype of a twin-layer PBR system composed of vertical biofilm sheets for the treatment of effluents from the secondary stage of municipal wastewater treatment with advanced nitrogen and phosphorus removal. Namely, effluents were collected from the bio-phosphorus anaerobic tank, the denitrification tank and the final settler, and the effect of phosphorus supplementation was tested. Effective nitrogen and phosphorus removal was achieved for all biofilm experiments, being phosphorus addition to the settler effluent unnecessary for efficient operation of the system. Also, the main nutrient removal mechanism was microalgae uptake, therefore, recovery and reuse of these nutrients is possible, towards the circular economy concept.

Gou et al. (2020) performed a long-term operation of an algal/bacterial biofilm reactor fed with synthetic wastewater using polyethylene carriers. Different HRT values were tested and the best operation considering the COD, nitrogen, and phosphorus removal yields occurred with HRT at 12 h, no improvement resulting from extending it to 24 h. Also, it is noteworthy that the algae consortium profile in the culture suffered changes concerning the dominant species when compared to the inoculum used.

Tao et al. (2017) also performed experiments using biofilm carriers, this time in a flat plate system. Filtered municipal wastewater was used in this study and higher nitrogen and phosphorus removal efficiencies were achieved when compared to a suspended biomass system operating in the same conditions. In terms of removal mechanisms, the dominant for nitrogen was microalgae uptake while precipitation, as well as biomass uptake, were relevant for phosphorus removal. It is noteworthy that although biofilm carriers were used, a high proportion of suspended microalgae culture was also present. This factor must be taken into consideration in the subsequent microalgae harvesting process.

Table 5
Published results for fixed microbial cultivation on wastewater in systems with self-attachment.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Flow cell system (0.36 L)	Sample form settling tank (mainly *Nitzschia* sp.)	Operation: 10 days HRT: 1.4 | 0.7 days 230 μmol m^{-2} s^{-1} L:D = 24:0; 22 °C CO_2; pH: 7	Secondary ef.	N–NO_3: 5.57 P–PO_4: 0.97	N–NO_3: 60 P–PO_4: 88	HRT = 1.4 5.5 g m^{-2} d^{-1} HRT = 0.7 3.0 g m^{-2} d^{-1}	Boelee et al. (2011)
Flow cell system (0.36 L)	Sample form settling tank (*Monoraphidium* sp. and *Scenedesmus acutus*)	Batch operation: 26 days HRT: 4.5 h 340 μmol m^{-2} s^{-1} L:D = 24:0; 23 °C	Synthetic wastewater	N–NH_4: 50 P–PO_4: 10 Acetate: 323	–	–	Boelee et al. (2014)
Horizontal Flat Panel PBR (HFP) (large scale)	Sample from sludge (*Phormidium autumnale*, *Pseudanabaena* sp. and *Scenedesmus acutus*)	Continuous operation: 2 × 9 months; HRT: 7 min 1.57–3.1 MJ m^{-2} d^{-1} L:D = 12:12;19–24 °C	Secondary ef.	TP: 2.9 TN: 49.4 N–NO_3: 48.6 N–NO_2: 0.23 N–NH_4: 0.33	12:12 | 24:0: TP: 36 | 97	12:12 | 24:0: 5.6 | 12.21 g m^{-2} d^{-1}	Sukačová et al. (2015)
Algal biofilm reactor (3 L)	Natural consortium (dominated by *Chlorella* and *Phormidium*)	Operation: 6 days	Domestic grey water (G) and anaerobically digested slurry (A)	G | A: COD: 235 | 2200 N–NO_3: 6 | 73 TDP: 25 | 257 N–NH_4: 30 | 254	G | A: COD: 70 | 80 N–NO_3: 100 | – TDP: 90 | 88 N–NH_4: 94.2 | 93	G | A: 3.6 | 3.1 g m^{-2} d^{-1}	Choudhary et al. (2017)
Open biofilm bioreactor (31 L)	Microalgal consortium and activated sludge from a WWTP	Operation:140 days HRT: 3.1(III) | 5.2(II) | 10.4(I) days 88 μmol m^{-2} s^{-1} L:D = 16:8; 19–25 °C	Primary ef.	TOC: 181 IC: 100 TN: 91 N–NH_4: 66 P–PO_4: 7 N–NO_3: 0 N–NO_2: 0	HRT = I | II | III: TOC: 90 | 86 | 86 TIC: 91 | 81 | 85 TN: 70 | 59 | 54 P–PO_4: 85 | 57 | 36	HRT = I | II | III: 2 | 3.1 | 2.6 g m^{-2} d^{-1}	Posadas et al. (2013)
Open biofilm PBR (31 L)	Mixed microalgal–bacterial consortium	Operation: 40 days HRT: 10 days 74 μmol m^{-2} s^{-1} L:D = 16:8; 20–25 °C	Secondary ef.	TOC: 167 IC: 122 TN: 106 P–PO_4: 12 N–NH_4: 86 N–NO_3: 0 N–NO_2: 0	TOC: 89 IC: 89 TN: 92 P–PO_4: 96	3.8 g m^{-2} d^{-1}	Posadas et al. (2014)
Twinlayer PBR (55 L)	*Halochlorella rubescens* CCAC 012	Batch operation: 32 days 22–220 μmol m^{-2} s^{-1} 18–32 °C	Secondary (with and without PO_4 addition), (S) and (SP) denitrification (D) and bio-phosphorus (Bio-P) ef.	S | SP: P–PO_4: 0.61 | 2 N–NO_3: 7.51 | 5.85 D | Bio-P: P–PO_4: 1.95 | 3.81 N–NH_3: 1.79 | 11.10	S | SP: P–PO_4: 73.2 | 70.4 N–NO_3: 83.2 | 82.9 D | Bio-P: P–PO_4: 84.8 | 78.9 N–NH_3: 95.5 | 99.4	6.3 g m^{-2} d^{-1}	Shi et al. (2014)
Algal-bacterial biofilm reactor (2 L)	Sample form settling tank and activated sludge from WWTP	Continuous operation:150 days HRT: 24 | 12 | 8 h 28 °C	Synthetic wastewater	COD: 300 N–NH_4: 30 P–PO_4: 10	HRT = (24 | 12 | 8): COD: 50–90*| 70–90*| 40–60* N–NH_4: 40–90*| 70–90*| 40–60* P–PO_4: 10–40*| 20–50*| 20*	–	Gou et al. (2020)

(*continued on next page*)

Another study using microalgae carriers was performed by He and Xue (2010), treating secondary effluent from a WWTP. During the 91-day operation period bacteria started to accumulate on the carriers improving COD removal and performing the nitrification of the ammonium present in the wastewater. The final concentrations of pollutants in the treated wastewater met the local regulatory discharge criteria.

Table 5 (*continued*).

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Flat-plate algal biofilm airlift PBR with algae carriers (20.3 L)	*Chlorella vulgaris*	Continuous operation: 37 days HRT: 10.15 days 120.8 μmol m^{-2} s^{-1} 25–30 °C; Air	Municipal (filtered)	TN: 16.43 DIP: 3.07 $N–NH_4^+$: 0.04 $N–NO_3^-$: 15.81 $N–NO_2^-$: <0.001 COD: 21.26 TP: 3.25 DIN: 15.86	DIN: 61.6 DIP: 71.3	Suspended: 11.8 mg L^{-1} d^{-1} Attached: 4.1 mg L^{-1} d^{-1} Total: 15.9 mg L^{-1} d^{-1} 0.82 g m^{-2} d^{-1}	Tao et al. (2017)
PBR with algae carriers (96 L)	*Scenedesmus* sp.	Continuous operation: 91 days HRT: 2 days 2800 lx; L:D = 12:12 20–22 °C	Secondary ef.	COD: 45–60 $N–NH_4$: 3.8–7.6 TN: 12.5–23.8 TP: 0.82–1.67	COD: 21–48 $N–NH_4$: 24–55 TN: 3 ∗ 6 TP: >39–70 (62*)	–	He and Xue (2010)
Revolving Algal Biofilm (RAB) (1000 L) Height: 0.9 m (A) | 1.8 m (B)	Algal consortium from the clarifiers of a WWTP	Semi-continuous operated: 180 days HRT: 1.3 | 4.7 | 7 days; 10–30 °C	Pre-treatment ef.	$N–NH_3$: 2–10* TP: 2–11* $P–PO_4$: 1–3* TKN: 13–28*	A (HRT = 1.3 | 4.7 | 7): TP: 44* | 62* | 56* $P–PO_4$: 40* | 80* | 96* TKN: 90* | 82* | 82* $N–NH_3$: 98* | 98* | 98* B (HRT = 1.3 | 4.7 | 7): TP: 64* | 90* | 90* $P–PO_4$: 68* | 96* | 99* TKN: 86* | 80* | 84* $N–NH_3$: 98* | 98* | 98*	A (HRT = 1.3 | 4.7 | 7): 4* | 3* | 1* g m^{-2} day^{-1} B (HRT = 1.3 | 4.7 | 7): 7* | 4.4* | 1.8* g m^{-2} day^{-1}	Zhao et al. (2018)
Rotating algal biofilm reactor (8 L)	Mixture of various algae strains	Fed-batch operation: 21 days HRT: 2 | 6 days 200 μmol m^{-2} s^{-1} L:D = 16:8; 21–25 °C	Secondary ef.	$N–NH_4$: 9.9 $N–NO_3$: 0.45 $N–NO_2$: 0.33 $P–PO_4$: 3.49 COD: 63.1 TKN: 49.2 TN: 50 TP: 15	HRT = (2 | 6) days $N–NH_4$: 92 | 100 $N–NO_3$: 44 | 47 $N–NO_2$: 57 | 58 $P–PO_4$: 97 | 79	HRT = 2 | 6 30.1 | 28 g m^{-2}	Shayan et al. (2016)
Rotating algal biofilm reactor (8 m^3)	–	Batch operation: 12 days HRT: 6 h 208 μmol m^{-2} day^{-1} 9.6–19.2 °C	Secondary ef.	$N–NH_3$: 7.8 TP: 4.5	TN: 76 TP: 23	377 g m^{-2} 31 g m^{-2} d^{-1}	Christenson and Sims (2012)

* Approximate values read from graphs.

A different type of system was operated by Zhao et al. (2018) for the treatment of wastewater from sludge thickening in a WWTP. A pilot scale open pond raceway was altered to include a revolving mechanism onto which algae biofilms attached, two height values for this revolving mechanism being tested. In this work different HRT values were used and a comparison with the conventional raceway configuration was performed. In general, the altered raceways performed better than the conventional ones in terms of nutrient removal. Comparing the two biofilm support heights, phosphorus removal was best for the tallest reactor, achieving around 90% removal at HRT of 7 and 4.7 days and 60% removal for HRT at 1.3 days, while the shorter reactor presented phosphorus removal yields between 40 and 80%. The total nitrogen removal performance was similar for all conditions tested, yields being always above 70%.

Shayan et al. (2016) performed experiments in a laboratory scale rotating algae biofilm reactor treating effluent from the secondary treatment stage of a WWTP, testing two HRT values (2 and 6 days). The results depended on the nutrient considered, nitrogen removal yield being higher with HRT at 2 days and phosphorus removal being best at 6 days. However, it is noteworthy that in both conditions the removal yield is high for both nutrients (above 79%). Christenson and Sims (2012) also performed tests using a rotating algae biofilm reactor for the treatment of a secondary effluent. However, lower nitrogen and phosphorus removal efficiencies were achieved. This result can be related to the use of a larger system as well as a much lower HRT (6 h).

Table 6
Published results for microalgae cultivation on wastewater in systems with biomass entrapment.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Ref.
PBR (alginate-immobilized microalgae) (200 mL)	*Chlorella vulgaris* AG30007	Batch operation: 3.5 days HRT: 3.5 days 100 μmol m^{-2} s^{-1}; L:D = 24:0 25 °C	Pre-treatment ef. (settling, centrifugation, filtration, autoclaved)	$N–NH_4$: 28.7 TP: 1.3	Activated sludge | algae: $N–NH_4$: 0 | 83 TP: 36 | 100 AS/A = 0.5 | 1 | 2 | 5 | 10: $N–NH_4$: 95 | 91 | 65 | 42 | 30 TP: 100 | 100 | 94 | 84 | 76	Mujtaba and Lee (2017)
Parallel-plate reactor (alginate entrapment as algal sheets) (350 mL)	*Scenedesmus* sp.	Operation: 21 days 5000 lux; L:D = 13:11 20 °C; Air	Secondary ef.	$N–NH_4$: 15.5 $P–PO_4$: 1.5	$N–NH_4$: 100 $P–PO_4$: 100	Zhang et al. (2008)
Conical flask (alginate beads) (2 L)	*Chlorella vulgaris*	Operation 48 h 180 μE m^{-2} s^{-1}; L:D = 16:8; 25 °C; Air	Primary ef.	–	$N–NH_4$: 100 $P–PO_4$: 95 $N–NO_3$: 96	Hameed (2007)
Glass container (alginate beads) (2.5 L)	Microalgae consortium from an HRAP	Operated: 10 days HRT: 2–4 days 150 μmol m^{-2} s^{-1}; L:D = 12:12; 23 °C	Secondary ef.	$N–NH_4$: 36.3 TP: 0.46	Free | Immobilized: $N–NH_4$: 64 | 89 TP: 90 | 96	Solé and Matamoros (2016)
CSTR (alginate beads) (3 L)	*Scenedesmus obliquus* (So) *Chlorella vulgaris* (Cv)	Batch (B) | semi-continuous (S) operation: 48 (B) | 250 (S) h 135 (B)| 200 (S) μE m^{-2} s^{-1} 25 °C; Air	Secondary ef.	$N–NH_4$: 32.5 $P–PO_4$: 2.5	B (So Free | Cv Free | *So* Imob. | Cv Imob.): $N–NH_4$: 100| 60.1| 96.6| 80.3 $P–PO_4$: 83.3| 55.2| 80.3| 53.3 S (So Imob.): $N–NH_4$: 10–90 $P–PO_4$: 18–64	Ruiz-Marin et al. (2010)
PVC bioreactor (alginate beads) (4 L)	*Chlorella vulgaris*	Batch | Continuous operation: 1–8 days HRT: 6.5 | 12 h 2 × 10^{15} quanta cm^{-2} s^{-1} L:D = 12:12; 20 | 30 °C; CO_2	Tertiary ef.	NOx: 5.67–8.69 $P–PO_4$: 0.08–1.78 BOD: 2–7 TP: 0.23–1.84 TKN: 1.09–3.39 TN: 7.10–10.36	Batch | Continuous: NOx: 100 | 100 $P–PO_4$: 80–90 | 70	Filippino et al. (2015)
Bubble columns (alginate beads) (5 L)	*Chlorella vulgaris*	Batch; Operation: 48 h 174 μE m^{-2} s^{-1}; L:D = 24:0; 23 °C; Air	Synthetic primary ef.	$N–NH_4$: 30 $P–PO_4$: 6	$N–NH_4$: 76–100 $P–PO_4$: 86.7–93.9	Tam and Wong (2000)
Aerated tubes (entrapment in alginate beads) (4 × 2.5 L)	*Chlorella minutissima*	Batch; Operation: 48 h 6480 lux; L:D = 12:12 25 °C; Air	Raw sewage	$N–NH_3$: 37 $P–PO_4$: 12.8 $N–NO_3$: 350 COD: 175	$N–NH_4$: >99 $P–PO_4$: >99 $N–NO_3$: 58 COD: 70	Singh et al. (2012)

2.2.2 Entrapment

Some studies have been performed on different bioreactor systems using microalgae entrapment, an overview being presented in Table 6. All the studies found in this literature survey were conducted at laboratory scale, a circumstance that apparently indicates that upscaling is a latent issue with this technology.

Mujtaba and Lee (2017) performed experiments to determine the ideal activated sludge/microalgae ratio for the optimal operation of a suspended sludge/alginate-entrapped microalgae mixed culture. First, it is noteworthy that the activated sludge single culture led to very low nitrogen and phosphorus removal yields, while the single culture microalgae system could achieve complete phosphorus removal and high (>83%) nitrogen removal levels. However, the use of mixed sludge and microalgae at a ratio of 1:2 allowed almost complete removal of both nutrients (>95%).

Zhang et al. (2008) immobilized the microalgae in alginate sheets in a parallel plate reactor and used it to treat the secondary effluent of a WWTP. The study achieved complete removal of nitrogen and phosphorus from the wastewater. Also, the algal sheets withstood the total period of the experiment (21 days) without deterioration. However, further scale up studies would need to be performed before the technology could be considered for large scale operation.

Hameed (2007) tested different alginate bead characteristics aiming to determine the best conditions for the removal of nitrogen and phosphorus from the primary effluent of a WWTP in batch reactors. It was determined that the optimal settings would be a bead size of 4 mm, a microalgae concentration of 1.5 x 10^6 cells/bead and a bead:wastewater volume

ratio of 1:3. In these conditions almost complete removal was achieved (>95%). The nitrogen removal mechanisms identified were mainly microalgae uptake and adsorption to the bead matrix.

Solé and Matamoros (2016) compared the nitrogen and phosphorus removal efficiencies achieved by two identical systems treating the secondary effluent of a WWTP, both inoculated with the same microalgae consortium, one in suspended form and another immobilized in alginate beads. While phosphorus removal was efficient in both systems (>90%), nitrogen removal was much higher for the immobilized system than for the suspended alternative (64 and 89%, respectively). The main processes identified for nitrogen removal were microalgae uptake and nitrification.

Ruiz-Marin et al. (2010) compared the treatment of a WWTP secondary treatment effluent by two microalgae strains (*Scenedesmus obliquus* and *Chlorella vulgaris*) testing both suspended and alginate immobilized cells. In the batch experiments performed, the free cells presented better nutrient removal efficiencies than the immobilized cells, being the best removal achieved by *Scenedesmus obliquus*. Semi-continuous operation was subsequently tested using the entrapped microalga giving best results in batch operation. Higher removal efficiencies were achieved than in batch operation, but still below those obtained using free cells.

Filippino et al. (2015) also operated a reactor with alginate immobilized microalgae fed with a WWTP tertiary treatment effluent, first in batch mode to test different temperature, illumination and pH control settings, and subsequently in continuous mode to determine the ideal HRT value. Complete nitrogen removal was achieved with an HRT of 6.5 h. In this work it was considered that nitrogen removal was mostly due to microalgae uptake.

Tam and Wong (2000) and Singh et al. (2012) operated similar bubble column systems with microalgae entrapped in alginate beads for the treatment of a synthetic primary effluent and raw sewage, respectively. In both studies high removal yields were attained for nitrogen and phosphorus, especially for the Singh et al. (2012) study (almost complete removal except for nitrate). For the Tam and Wong (2000) experiments it was concluded that besides nutrient uptake by the microalgae, ammonia volatilization and phosphorus precipitation also occurred.

2.3 Alternative and combined technologies

The systems reviewed in the previous chapters employ one specific kind of technology, however reports have also been published involving technology alterations, and in which different technologies are combined in an attempt to keep the advantages and avoid the limitations of each individual one. An overview of studies carried out using these alternative configurations is presented in Table 7.

Since one of the typical major limitations of microalgae culturing in photobioreactors is light supply, Xue et al. (2013) introduced optical fibre to an airlift flat panel PBR, thus improving the irradiance level per cell. The inclusion of optical panels in flat systems has also been tested, originating the optical flat plate PBR (OPPBR). Choi (2014) performed experiments in an OPPBR in order to determine the impact of the distance between optical panels (therefore the culture depth covered by each) on the treatment of an effluent from a WWTP preliminary stage. The distance promoting the best nutrient removal (considering COD, TN, $N–NH_4$, TP, and phosphate-phosphorus ($P–PO_4$)) was 112.5 mm. Choi and Lee (2015) also performed experiments using the same OPPBR and the same wastewater source to determine the best nitrogen/phosphorus ratio in the feed for both biomass productivity and wastewater remediation. The N/P ratio promoting the highest nitrogen removal was 11/20 while that giving the best performance in phosphorus removal and biomass production was 1/10. In the latter case, nitrogen removal was still quite substantial (78.35%).

One of the technologies receiving most attention from the scientific community in the context of wastewater treatment with microalgae is the membrane reactor, which can be easily combined with many reactor types among those reviewed already in previous sections. Thus, Choi (2015) combined the OPPBR with a membrane bioreactor (MBR) proposing the microalgae membrane bioreactor (MMBR). The same preliminary effluent from this author's work mentioned in the above paragraph was used to test the efficiency of this new combination. The results obtained are promising, more than 90% removal being reported for all the nutrients analysed.

Singh and Thomas (2012) and Wang et al. (2013b) both operated a system combining an activated sludge process with a microalgae cultivation process, with two membrane reactors, one following the other. Singh and Thomas (2012) performed batch experiments with various microalgae species achieving good removal efficiencies for *Chlorella vulgaris*. However, a subsequent continuous operation with this microalga resulted in much lower nutrient removal yields. Wang et al. (2013b) focused mainly on the microalgae biomass production yield when fed with the outlet of the activated sludge bioreactor. The results show that nitrifying bacteria compete with the growth of the microalga being cultivated, reducing biomass productivity, and thus the operation of two sequential MBR, excluding nitrifiers from the microalgae culture feed, would be beneficial.

Gao et al. (2015) combined a flat plate PBR with a membrane reactor and the biofilm technology in order to treat a synthetic secondary effluent. The nutrient removal efficiencies obtained were high (>80%), and since the harvesting of the microalgae is already provided by the membrane in the system, biomass use for other applications is simplified and the treated water can be discharged with no further processing. Also, it is noteworthy that ammonia volatilization and phosphorus precipitation were reduced, and microalgae uptake was the main nitrogen and phosphorus removal mechanism. Gao et al. published two other works (Gao et al., 2014, 2016) where a column reactor was combined with membrane modules for the treatment of real domestic wastewater pre-treated at secondary level. The removal yields achieved were sufficient to meet regulatory wastewater discharge limits, and the main nitrogen removal mechanism was microalgae uptake. Phosphorus precipitation occurred along with microalgae assimilation in the Gao et al. (2014) study.

Table 7
Published results for different alternative or combined systems being developed for microalgae cultivation on wastewater.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Optical panel PBR (OPPBR) (37 L)	*Chlorella vulgaris*	Batch Operation: 10 days 100–200 μmol m^{-2} s^{-1} L:D = 16:8; 23 °C; Air	Preliminary ef.	TCOD: 185–255 TN: 33.5–49.2 $N–NH_4$: 24.8–43.5 TP: 15.1–35.6 $P–PO_4$: 5.1–11.2 BOD: 125–181 $N–NO_3$: 16.4–28.7	Distance (225| 150| 112.5| 90) mm: COD: 73.2| 84.5| 88.4 | 87.2 TN: 78.4 | 88.4 | 96.5 | 90.5 $N–NH_4$: 96.8 | 98.6 | 99.1 | 98.1 TP: 69.5| 79.7| 84.6| 80.2 $P–PO_4$: 69.1| 78.4| 84.1| 80.0	Distance (225| 150| 112.5| 90) mm: 11.24 | 12.04 | 12.56 | 11.87 g/L	Choi (2014)
Optical panel PBR (OPPBR)	*Chlorella vulgaris* (FC-16)	Batch operation: 15 days 270 and 310 μE m^{-2} s^{-1} L:D = 16:8; 25 °C; Air	Preliminary ef.	TN: 33.53–49.24 TP: 5.07–15.58	N:P ratio (1:10| 11:20 | 21:30| 31:40| 41:50| 51:60| 61:70): TN: 78.35 | 83.90 | 82.81 | 78.08 | 73.29 | 72.40 | 72.50 TP: 88.54 | 80.98 | 59.00 | 44.15 | 34.00 | 23.10 | 23.90	N:P ratio (1:10| 11:20 | 21:30| 31:40| 41:50| 51:60| 61:70): 2.75 | 2.3 | 1.18 | 0.78 | 0.43 | 0.40 | 0.41 g L^{-1} d^{-1}	Choi and Lee (2015)
Microalgae membrane bioreactor (MMBR) (combining the OPPBR and MBR)	*Chlorella vulgaris*	Batch operation: 150 days HRT: 3.4 days (72 h OPPBR | 9 h MBR) 270–310 μE m^{-2} s^{-1} L:D = 17:8; 25 °C; Air	Preliminary ef.	BOD_5: 125–180 TCOD: 185–255 TN: 33.53–49.24 $N–NH_4$: 20.8–33.5 $N–NO_3$: 10.4–18.7 TP: 5.1–15.6 $P–PO_4$: 5.1–11.2	BOD: 97.09 COD: 96.99 TN: 96.38 $N–NH_4$: 99.80 $N–NO_3$: 97.62 TP: 92.75 $P–PO_4$: 90.84	2.53 g L^{-1} day^{-1}	Choi (2015)
Flat panel microalgae membrane PBR (5 L | 10 L)	*Chlorella* sp., *Chlorella vulgaris, Scenedesmus quadricauda* and *Scenedesmus dimorphus*	Batch | Continuous operation: 23 days HRT: 1.6 days 4000 lux; L:D = 12:12 24 °C; Air	Permeate from activated sludge membrane bioreactor (MBR)	$N–NH_4$: 0.7–1.4 $N–NO_3$: 50–80 $N–NO_2$: 18–25 $P–PO_4$: 10–20	Batch every species | Continuous *C. vulgaris*: $N–NH_4$: 100 | 50 $N–NO_3$: 43–54 | 35 $N–NO_2$: 83–95 | 75 $P–PO_4$: 70–92 | 60	–	Singh and Thomas (2012)
Dual MBR (sequential bacteria–microalgae)	*Chlorella vulgaris* Beij.	Continuous operation: 18 days Air	Primary ef. (Autoclaved | not autoclaved)	$N–NO_3$: 44.0	Not autoclaved | Autoclaved: $N–NO_3$: 10 | 22	–	Wang et al. (2013b)
Flat-plate algal biofilm membrane photobioreactor (BMPBR)	*Chlorella vulgaris*	Continuous operation: 20 days HRT: 2 days 8000 lux; 25–28 °C Air+4% CO_2	Synthetic secondary ef.	$N–NH_4$: 5.0 TIN: 15.0 $P–PO_4$: 0.8	$N–NH_4$: 96 TIN: 82.5 $P–PO_4$: 85.9	0.072 g L^{-1} d^{-1}	Gao et al. (2015)
Columnar membrane PBR (MPBR)	*Chlorella vulgaris*	Continuous operation: 35 days HRT: 2 days 120.8 μmol $m^{-2}s^{-1}$ 25–30 °C; Air+4% CO_2	Secondary ef.	TN: 14.12 $P–PO_4$: 0.72	TN: 87.7 $P–PO_4$: 76.7	50.72 mg L^{-1} d^{-1}	Gao et al. (2016)

(*continued on next page*)

Table 7 (*continued*).

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Columnar PBR equipped with submerged MF membrane module	*Chlorella vulgaris*	Batch operation: 15 days HRT: 2.5 days 8000 lux; 25 °C; Air	Plant ef.	TN: 19.12 TP: 1.24 COD: 55.6 N–NH_4: 11.26 N–NO_3: 7.06 N–NO_2: 0.15	TN: 50.1–62.3 TP: 72.6–91.9	39.93 mg L^{-1} d^{-1}	Gao et al. (2014)
Osmotic membrane PBR (OMPBR) (5.5 L)	*Chlorella vulgaris*	Continuous operation: 162 days HRT: 2, 3, 4 days 1000–1500 lux Air+5% CO_2	Synthetic secondary (S) tertiary (T) ef.	Secondary | Tertiary N–NH_4: 22 | 8 N–NO_3: 0.8 | 0.8 P–PO_4: 6 | 2.4	N–NH_4: >90 N–NO_3: >50 P–PO_4: >85	>5 g/L	Praveen and Loh (2016)
Membrane PBR (MPBR) | osmotic membrane PBR (OMPBR) (5.5 L)	*Chlorella vulgaris*	Operation: 12 (MPBR) | 16 (OMPBR) days HRT: 2 days 1500–2000 lux Air+5% CO_2	Secondary ef.	N–NH_4: 4 mg/L P–PO_4: 1.8	OMPBR: N–NH_4: 92–99 P–PO_4: 100 MPBR: N–NH_4: 84–97 P–PO_4: 28–47	>2 g/L	Praveen et al. (2016)
Double column-type reactor with submerged membrane (10L)	*Chlorella* sp. ADE4 and *Chlorella vulgaris*	Batch (B) | Continuous (C) Operation: 7 (B) | 18 (C) days HRT: 2 days (C) 50 μmol m^{-2} s^{-1} L:D = 14:10; 25 °C; Air	Secondary ef.	TN: 18.8 TP: 1.01	Chlorella sp.: TN: 67.5 (B) | 66.5 (C) TP: 100 (B) 94.5 (C) Chlorella vulgaris: TN: 63.6 (B) TP: 78.5 (B)	*Chlorella* sp. (B): ∼400–500 mg/L Chlorella v. (B): ∼100 mg/L *Chlorella* sp. (C): 55 mg L^{-1} d^{-1} 1300 mg/L	Boonchai and Seo (2015)
Batch wise PBR | Sequencing batch membrane PBR (SB-MPBR)	Dominated by *Euglena* sp. from an open pond near the WWTP	PBR Batch operation: 14 days SB-MPBR HRT: 2 | 4 | 8 days BRT: 60 days 10 000 lux; Air	Secondary ef. (filtered)	TN: 24.7 TP: 3.5	PBR: TN: 96.76 TP: 37.14 SB-MPBR (HRT = 2 | 4 | 8): TN: 82.79 | 95.95 | 70.00 TP: 35.71 | 90.28 | 44.29	PBR | SB-MPBR (HRT 2 | 4 | 8): 515 | 580 | 710 | 1000 mg/L 36.429 | 10.500 | 11.833 | 16.667 × 10^3 kg m^3 d^{-1}	Sheng et al. (2017)
Photo-sequencing batch reactor (PSBR)	Microalgal–bacterial consortium developed naturally	Operation: 6 months HRT: 2 days 30 μmol m^{-2} s^{-1} L:D = 16:8; 22.8 °C	Primary ef.	COD: 257 TKN: 55 TP: 4.8	COD: 86 TKN: 97 TP: 47	–	Foladori et al. (2018)
Photo-sequencing batch reactor (PSBR) (1.5 L)	Mixture of *Scenedesmus obliquus* and activated sludge	Operation: 30 days HRT: 3 h cycle duration 54 μmol m^{-2} s^{-1} L:D = 24:0; 24 °C; Air	Domestic (filtered)	COD: 189 TN: 26 N–NH_3: 24 TP: 6.2	COD: 72 TN: 20 N–NH_3: 72 TP: 5	7400 mg/L	Purba et al. (2021)

(*continued on next page*)

Praveen and Loh (2016) performed a long-term operation with an osmotic membrane PBR treating synthetic wastewater. The removal yield values achieved were high (above 85% for ammonia nitrogen and phosphate, and above 50% for nitrate) during the whole operation. Praveen et al. (2016) also performed experiments to compare the removal efficiencies achieved by the osmotic membrane PBR and a microfiltration PBR. The forward osmosis membrane allowed better nitrogen removal and much higher phosphorus removal yields when compared to the microfiltration membrane.

Table 7 (*continued*).

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Semi-open system (1500 L)	*Chlorella* sp.	Batch(B)| Semi-continuous(S) operation: 13 (B) | 17–23 (S) days HRT: 5.6–7.6 (S) days 25 μmol m^{-2} s^{-1}; 25 °C CO_2| No CO_2	Centrate	TP: 392 TKN: 275 $N–NH_3$: 113 COD: 3027	Batch | Semi-continuous: TP: 58.1 | 61 TKN: 34.8 | 61 $N–NH_3$: 19.5 COD: 86.3 | 70	34.6 g TSS m^{-2} day^{-1} and 17.7 g VSS m^{-2} day^{-1}	Min et al. (2011)
Offshore floating membrane PBR (Tubular) (110 L)	Green microalgae mixture dominated by *Desmodesmus* sp.	Continuous operation: 23 days Flue gas	Secondary ef.	–	$N–NH_3$: >90	14.1 g dry bio-mass m^{-2} day^{-1}	Wiley et al. (2013)
Enclosed, offshore, floating PBRs (12–48 × 4.18–20.91 m^3)	*Scenedesmus dimorphus* inoculum replaced by natural consortium	Continuous operation: 1 year 250–600 μmol m^{-2} s^{-1} 10–30 °C; Air+CO_2	Primary ef. (filtered and disinfected)	$N–NH_3$: 26.5 TN: 40 $P–PO_4$: 2.98 TP: 4.22 COD: 542 BOD: 300	$N–NH_3$: 80 TN: 75 $P–PO_4$: 95 TP: 93 COD: 84 BOD: 92	3.5–22.7 g m^{-2} day^{-1}	Novoveská et al. (2016)

Boonchai and Seo (2015) used a double column PBR combined with a membrane module to treat a secondary effluent. Two strains of *Chlorella* were tested, batch experiments being first performed to identify which of the two achieved higher nutrient removal yields (especially phosphorus). Then, only the best strain was used in a continuous mode experiment, resulting in slightly lower removal yield values but in much higher biomass productivity.

The sequencing batch reactor, typically used with activated sludge, was also tested by different authors for microalgae cultivation in wastewater. Sheng et al. (2017) compared the operation of a typical PBR and a sequencing membrane PBR, using different HRT values, in the treatment of a secondary effluent. Nitrogen removal was high in the simple PBR, but phosphorus removal was very low. Nutrient removal in the sequencing membrane PBR was found to be highly dependent on the HRT value, best results being achieved for 4 days, with removal yield values above 90% for both nitrogen and phosphorus.

A long-term operation with a photo-sequencing batch reactor was performed by Foladori et al. (2018), where real primary effluent from a WWTP was treated with a spontaneous microalgae–bacterial consortium. High COD and nitrogen removal yields were achieved (both >80%) while phosphorus removal was low. It is noteworthy that the major nitrogen removal mechanism was autotrophic nitrification.

Purba et al. (2021) also used a photo-sequencing batch reactor for the treatment of domestic wastewater developing microalgae–bacteria granular sludge from the initial reactor inoculum. Although the system was efficient in COD removal, nitrogen and phosphorus removal yields were quite low, except for ammonia nitrogen.

Wiley et al. (2013) operated an offshore membrane tubular PBR to treat a secondary effluent with a microalgae mixed culture. An efficient nitrogen removal and biomass production performance was achieved. Novoveská et al. (2016) also operated offshore, however a larger scale system was built, composed of 12 to 48 PBRs. A yearlong continuous operation was performed, achieving efficient nutrient removal.

3 Nutrient mass balances in recent reports on wastewater treatment with microalgae

Carbon, nitrogen, and phosphorus contents are monitored in the effluents from WWTPs to avoid contamination of the natural water bodies into which the treated wastewater is discharged. However, to determine the fate of these elements in the process, detailed mass balances are required, measuring their content in each of its outputs. This information is useful to allow the shift in operational options towards element recovery, namely minimizing the fractions emitted to the atmosphere. Introducing one of more microalgae cultivation steps can be one of these operational options. They can be introduced in the conventional wastewater treatment process at different locations, namely intercepting the outlets (effluents) from the pre-treatment, from primary, secondary or tertiary treatment stages, or even from the anaerobic digestion of sludge (centrate from digested sludge dewatering). Fig. 1 shows the main operational stages of a classical wastewater treatment plant, including the water processing line and the stabilization stage (anaerobic digestion) from the sludge processing line, identifying the intermediate streams (effluents and centrate) that could be diverted for microalgae cultivation. These possibilities have all been addressed in the studies here reviewed, but only the options using secondary or tertiary effluents could be expected to produce water streams complying with regulatory limits for discharge into natural water bodies. However, all options or even combinations among them can be envisaged as contributing for a

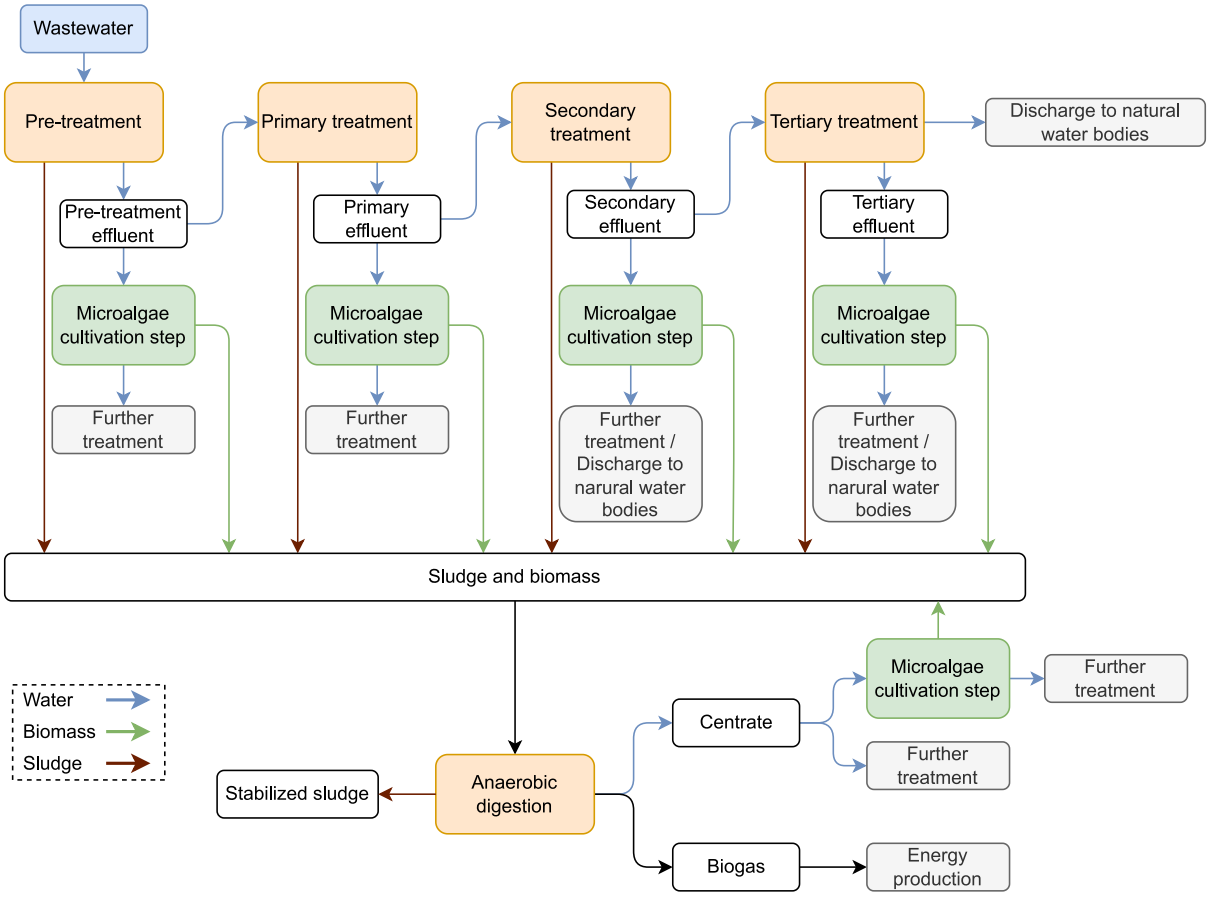

Fig. 1. Schematic overview of the possibilities of introducing a microalgae cultivation step in the classical wastewater treatment process. Here, anaerobic digestion of the harvested microalgae biomass is suggested. Further treatment of the water streams generally involves reintroduction into the main treatment line at the appropriate stage.

circular economy concept, through converting more of the C, *N* and P resources to recoverable biomass. The concept of Fig. 1 shows the microalgae biomass streams being entirely directed to anaerobic co-digestion with the other WWTP sludge streams. This option circumvents the varying quality and public health issues associated to other uses of this biomass and, moreover, opens possibilities for improving the productivity and quality of the bioenergy (biogas) and biofertiliser (stabilized sludge) outputs of the WWTP. To assess these possibilities, proper mass balances, as well as energy production and demand quantification, are needed.

The most reported carbon and nutrient removal mechanisms occurring during microalgae cultivation on wastewater are uptake by biomass (both microalgae and bacteria), volatilization of nitrogen (ammonia and the gaseous products of denitrification) and carbon to the atmosphere, and precipitation of phosphorus and inorganic carbon. Nitrification, whereby ammonium is converted to nitrate and nitrite, can also occur and affect nitrogen uptake by the microalgae. Also, different nitrogen forms can be assimilated by microalgae, depending on the strain, ammonia typically being the preferential form, and therefore the first to be removed (nitrate, nitrite and organic nitrogen forms are assimilated only after reduction of the ammonia concentration). The use of natural (spontaneous) mixed microalgae populations favours their capacity to promptly adapt to different N-source profiles in the feed effluent.

To perform complete nitrogen mass balances, minimum measurements include total nitrogen and the nitrate and nitrite ($N–NO_2$) nitrogen fractions, although quantification of the ammonia nitrogen and organic nitrogen fractions can be useful for the understanding of the involved conversion mechanisms. For phosphorus and carbon mass balances, minimum analyses are total phosphorus and total carbon, the latter generally providing information on total inorganic (TIC) and total organic carbon fractions. COD and BOD are often monitored in some studies but typically are not used to perform elemental mass balances. Table 8 presents these removal or conversion routes, the outputs carrying the involved elements and their forms, and the analyses that are required to quantify their impact and perform the mass balances. Fig. 2 presents illustrations of the routes here covered.

It is noteworthy that most studies on wastewater treatment with microalgae do not perform detailed mass balances to the main elements (nitrogen, phosphorus, and carbon), their removal mechanisms being sometimes just assumed or

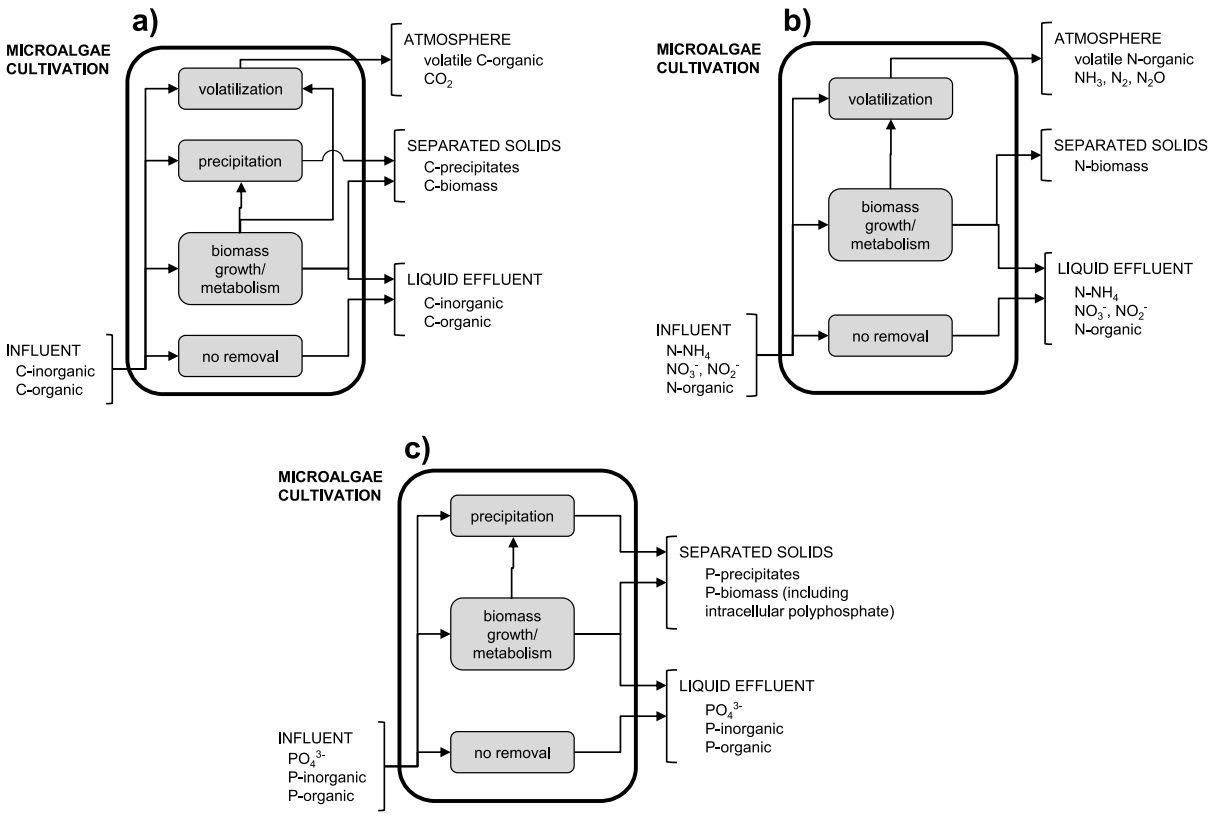

Fig. 2. Schematic overview of the main routes of carbon (a), nitrogen (b) and phosphorus (c) compound conversion which can occur in open microalgae cultivation on urban wastewater.

Table 8
Main routes for removal or conversion of nitrogen, phosphorus and carbon taking place in microalgae cultivation, the produced outputs and forms of the nutrients in them, and the analyses required to perform mass balances. The designations %C, %N and %P refer to elemental analysis of the dry biomass. NA — not applicable.

Element	Biomass uptake		Volatilization		Precipitation		Nitrification		No removal	
	Output (form)	Analyses required	Output (form)	Analyses required	Output (form)	Analyses required	Output (form)	Analyses required	Output (form)	Analyses required
Nitrogen	Harvested biomass (N-organic)	Biomass dry weight; %N	Emissions to the atmosphere (NH_3, N_2, N_2O, other volatiles)	NA	NA	NA	Treated water (NO_3^-, NO_2^-)	N–NO_3 N–NO_2	Treated water (NH_4, NO_3, NO_2, N-organic)	TN N–NH_4 N–NO_3 N–NO_2 N-organic
Phosphorus	Harvested biomass (P-organic; polyphosphate)	Biomass dry weight; %P	NA	NA	Harvested biomass (PO_4^{3-})	Biomass dry weight; %P	NA	NA	Treated water (PO_4, P-organic)	TP P–PO_4
Carbon	Harvested biomass (C-organic)	Biomass dry weight; %C	Emissions to the atmosphere (CO_2, other volatiles)	NA	Harvested biomass (CO_3^{2-})	Biomass dry weight; %C	NA	NA	Treated water (C-organic, C-inorganic)	TC TIC TOC

proposed. From the 119 research articles considered in this review only 9 presented the data required to perform nitrogen mass balances, while only 4 reported data for phosphorus and 2 for carbon. Typically, no elemental analyses are performed on the produced biomass impairing the estimates of nutrient uptake through microalgae growth. Also, in many studies only the orthophosphate form of phosphorus is considered and not total phosphorus, and the nitrate and nitrite forms of nitrogen are not measured, not allowing the impact of nitrification to be determined. The lack of measurements required for the balance on carbon may be due to the main objective of some studies being biomass production and not wastewater treatment, therefore not considering carbon recovery as a beneficial process to be duly quantified.

Table 9
Published results for microalgae cultivation on wastewater and calculated mass balances for nitrogen, phosphorus, and carbon. Biomass production and composition values are given on a dry weight basis.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Effluent pollutant load (mg/L)	Biomass production (mg/L)	Biomass composition (%)	Nutrient mass balance (%)			Ref.
									Effluent	Biomass	Other	
Borosilicate bioreactor 2 L	*Chlorella vulgaris*	Batch; Operation: 12 days 150 μmol m^{-2} s^{-1}; L:D = 14:10 20 °C ; Air; pH: 7.1–8.1	Pre-treatment ef.	TP: 8.91 TN: 88.5 N/P: 9.93	TP: 91.6 TN: 89.9	TP: 0.75 TN: 8.91	1388	N: 6[a] P: 0.5[a]	N: 10.1 P: 8.4	N: 94.4 P: 78.1	N: −4.5 P: 13.5	Cabanelas et al. (2013b)
			Pre-treatment ef.	TP: 8.81 TN: 52.1 N/P: 5.91	TP: 92.6 TN: 91.5	TP: 0.65 TN: 4.44	1404	N: 5[a] P: 0.9[a]	N: 8.5 P: 7.4	N: 118 P: 126	N: −27.0 P: −33.0	
			Primary ef.	TP: 5.08 TN: 35.6 N/P: 7.01	TP: 92.1 TN: 93.4	TP: 0.40 TN: 2.36	667.8	N: 7.4[a] P: 0.5[a]	N: 6.6 P: 7.9	N: 212 P: 100	N: −119 P: −8.3	
			Secondary ef.	TP: 9.07 TN: 64.1 N/P: 7.07	TP: 96.6 TN: 97.3	TP: 0.31 TN: 1.74	1538.4	N: 3.8[a] P: 0.8[a]	N: 2.7 P: 3.4	N: 70.7 P: 105	N: 26.6 P: −8.6	
			Disposing ef.	TP: 2.72 TN: 34.6 N/P: 12.7	TP: 88.2 TN: 55.5	TP: 0.32 TN: 15.4	957.8	N: 4.9[a] P: 0.4[a]	N: 44.5 P: 11.8	N: 93.4 P: 97.1	N: −38.0 P: −8.8	
			Disposing ef.	TP: 0.75 TN: 9.79 N/P: 13.1	TP: 80.0 TN: 89.5	TP: 0.15 TN: 1.03	886.6	N: 3.8[a] P: 0.2[a]	N: 10.5 P: 20.0	N: 276 P: 190	N: −187 P: −110	
			Centrate	TP: 60.5 TN: 130.1 N/P: 2.15	TP: 22.6 TN: 90.4	TP: 46.8 TN: 12.5	2341.2	N: 4.1[a] P: 4.7[a]	N: 9.6 P: 77.4	N: 33.8 P: 83.4	N: 56.6 P: −61.0	
Raceway 200 L	*Chlorella sp.*	Operation: 10 days 1711 μmol E m^{-2} s^{-1} 9.7–24–6 °C; Air	Primary effluent	TN: 22.7 TP: 9.8–11.6 N/P: 1.96–2.31	TN: 94.3–94.9 TP: >97	TN: 1.16–1.27 TP: 0–0.29	469[a]	N: 4.8 P: 0.3	N: 5.1 P: 0–3	N: 97.5 P: 10.9–12.9	N: −2.6 P: 84.1–89.1	Das et al. (2019)
	Scenedesmus sp.						457[a]	N: 5.1 P: 0.4	N: 5.7 P: 0–3	N: 100 P: 13.8–16.3	N: −5.7 P: 80.7–86.2	
Flat panel PBR 4.5 L	*Scenedesmus obliquus*	Continuous; HRT: 0.5 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2	Secondary effluent	TN: 16.6 TP: 2.00 N/P: 8.3	TN: 0 TP: 0	TN: 16.6 TP: 2.00	0	N: 0 P: 0	N: 100 P: 100	N: 0 P: 0	N: 0 P: 0	Ruiz et al. (2013)
		Continuous; HRT: 1.1 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2		TN: 19.7 TP: 1.75 N/P: 11.3	TN: 89.8 TP: 94.9	TN: 2.00 TP: 0.09	385	N: 5.90 P: 0.52	N: 10.2 P: 5.14	N: 115 P: 114	N: −25 P: −20	
		Continuous; HRT: 1.7 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2		TN: 22.2 TP: 2.14 N/P: 10.4	TN: 91.0 TP: 96.7	TN: 2.00 TP: 0.07	612	N: 5.00 P: 0.38	N: 9.01 P: 3.27	N: 138 P: 109	N: −47 P: −12	
		Continuous; HRT: 2.3 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2		TN: 15.2 TP: 0.81 N/P: 18.8	TN: 88.8 TP: 90.1	TN: 1.70 TP: 0.08	644	N: 3.30 P: 0.25	N: 11.2 P: 9.88	N: 140 P: 199	N: −51 P: −109	
		Continuous; HRT: 2.8 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2		TN: 34.9 TP: 3.56 N/P: 9.80	TN: 86.8 TP: 97.8	TN: 4.60 TP: 0.08	1064	N: 3.10 P: 0.11	N: 13.2 P: 2.25	N: 94.5 P: 32.9	N: −7.7 P: 64.9	
		Continuous; HRT: 3.4 days; 20 °C 250 μmol m^{-2} s^{-1}; L:D = 14:10 Air+5% CO_2		TN: 17.7 TP: 1.57 N/P: 11.3	TN: 80.8 TP: 94.9	TN: 3.40 TP: 0.08	986	N: 3.30 P: 0.18	N: 19.2 P: 5.10	N: 184 P: 113	N: −103 P: −18	
Open biofilm bioreactor 31 L	Microalgal consortium and activated sludge from a WWTP	Operation:140 days HRT: 10.4 days; 19–25 °C 88 μmol m^{-2} s^{-1}; L:D = 16:8	Primary effluent	TC: 281 TN: 91 $N–NO_3$: 0 $N–NO_2$: 0	TN: 70 TC: 90.4	TN: 27.3 $N–NO_x$: 19.1 TC: 27.1	–	N: 7 C: 42	N: 30.0 C: 9.6	N: 25.2 C: 45.2	N: 44.8 C: 45.2	Posadas et al. (2013)
		Operation:140 days HRT: 5.2 days; 19–25 °C 88 μmol m^{-2} s^{-1}; L:D = 16:8			TN: 59 TC: 84.2	TN: 37.3 $N–NO_x$: 31 TC: 44.3	–		N: 41.0 C: 15.8	N: 21.8 C: 40.4	N: 37.2 C: 43.8	
		Operation:140 days HRT: 3.1 days; 19–25 °C 88 μmol m^{-2} s^{-1}; L:D = 16:8			TN: 54 TC: 85.6	TN: 41.9 $N–NO_x$: 51.5 TC: 31.8	–		N: 46.0 C: 14.4	N: 16.2 C: 28.3	N: 37.8 C: 57.4	

(*continued on next page*)

We performed tentative mass balances for the studies reporting sufficient data to render this calculation possible and the results are presented in Table 9, together with authors' own calculations (Posadas et al., 2013, 2014, 2015). From the routes identified in Table 8, biomass uptake and precipitation are lumped together in the biomass fraction of Table 9, and

Table 9 (*continued*).

Open biofilm PBR 31 L	Microalgal–bacterial consortium	Operation: 40 days HRT: 10 days; 20–25 °C 74 μmol m^{-2} s^{-1}; L:D = 16:8	Secondary effluent	TC: 289 TN: 106	TC: 89 TN: 92	TC: 31.8 TN: 8.48	–	N: 8.9 C: 46.4	N: 8.0 C: 11.0	N: 49.3 C: 89.0	N: 42.7 C: 0.0	Posadas et al. (2014)
Raceway 700 L		Semi-continuous; 300–468 W m^{-2} Operation: 29 days; HRT: 2.7 days pH: 9; 13–23 °C; CO_2			TN 64.1 TC: 62.8	TN: 23 TC: 122	–	N: 8.7 C: 42.9	N: 35.9 C: 37.2	N: 51.9 C: –	N: 12.2 C: –	
Raceway 800 L		Semi-continuous; 300–468 W m^{-2} Operation: 29 days; HRT: 2.7 days pH: 8; 13–23 °C; CO_2		TN: 64 TC: 328	TN: 68.8 TC: 56.7	TN: 20 TC: 142	–	N: 8.9 C: 43.9	N: 31.3 C: 43.3	N: 58.4 C: –	N: 10.3 C: –	
Raceway 850 L		Semi-continuous; 300–468 W m^{-2} Operation: 29 days; HRT: 2.7 days pH: 7; 13–23 °C; CO_2			TN: 60.9 TC: 58.2	TN: 25 TC: 137	–	N: 6.4 C: 37.5	N: 39.1 C: 41.8	N: 41.4 C: –	N: 19.5 C: –	
Raceway 700 L	*Scenedesmus sp.*	Semi-continuous; 300–468 W m^{-2} Operation: 18 days; HRT: 2.8 days pH: 9; 13–23 °C; Flue gas	Primary effluent		TN: 53.8 TC: 72.2	TN: 24 TC: 126	–	N: 12.6 C: 50.4	N: 46.2 C: 27.8	N: 39.8 C: –	N: 14.0 C: –	Posadas et al. (2015)
Raceway 800 L		Semi-continuous; 300–468 W m^{-2} Operation: 18 days; HRT: 2.8 days pH: 8; 13–23 °C; Flue gas		TN: 52 TC: 453	TN: 71.2 TC: 68.4	TN: 15 TC: 143	–	N: 10.1 C: 61.5	N: 28.8 C: 31.6	N: 43.4 C: –	N: 27.8 C: –	
Raceway 850 L		Semi-continuous; 300–468 W m^{-2} Operation: 18 days; HRT: 2.8 days pH: 7; 13–23 °C; Flue gas			TN: 57.7 TC: 70.4	TN: 22 TC: 134	–	N: 9.5 C: 52.8	N: 42.3 C: 29.6	N: 34.6 C: –	N: 23.1 C: –	
Raceway 700 | 800 | 850 L		Semi-continuous; 300–468 W m^{-2} Operation: 32 days; HRT: 6.7 days pH: 8; 13–23 °C; Flue gas		TN: 75 TC: 378	TN: 82.2 TC: 59.3	TN: 13.3 TC: 154	–	N: 10 C: 62.9	N: 17.8 C: 40.7	N: 40.9 C: –	N: 41.4 C: –	
Raceway 700 | 800 | 850 L		Semi-continuous; 300–468 W m^{-2} Operation: 18 days; HRT: 6 days; 13–23 °C		TN: 70 TC: 313	TN: 97.1 TC: 49.3	TN: 2 TC: 159	–	N: 8.63 C: 51.5	N: 2.86 C: 50.7	N: 32.3 C: 39.4	N: 64.8 C: 9.9	
Raceway 530 L	*Scenedesmus obliquus*	Continuous; Operation: 157 days HRT: 10 days; pH: 8–9.5	Secondary effluent	TN: 24.92–26.16	TN: 65.12	TN: 8.69–9.12	299.1	N: 4.5–5	N: 34.9	N: 54–57.2	N: 8.0–11.1	Arbib et al. (2013)

(*continued on next page*)

the non-removed and nitrified fractions are those quantified as the effluents in Table 9. The mass balance result is given in Table 2 as other routes, which would comprise the emissions to the atmosphere and the precipitates not included in the biomass fraction.

Using the values published by Cabanelas et al. (2013b), Das et al. (2019) and Ruiz et al. (2013) the nitrogen and phosphorus mass balances result in negative values for the other routes, i.e., more nitrogen and phosphorus are present in the quantified outputs (biomass and effluent) than in the influent to the process. This may be due to the uncertainty of reading values from graphs and the use of different calculations for biomass productivity (equations not presented in the original reports).

Posadas et al. performed nitrogen and carbon balances in the three studies here considered (Posadas et al., 2013, 2014, 2015). It is noteworthy that the carbon mass balances of the Posadas et al. (2014) study as performed by the authors did not include the CO_2-carrying aeration flowrate value, thus not allowing complete balance calculations for carbon. The Arbib et al. (2013) and Su et al. (2012a) studies allowed only nitrogen mass balances, while the results from Zhao et al. (2018) could only be used for phosphorus mass balances.

Table 9 (*continued*).

Stirred tank PBR 14 L	Sample from the secondary clarifier (mainly filamentous blue-green algae) and activated sludge	Operation: 14 days 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 1:0	Primary effluent	TN: 50.1 $N\text{-}NO_3$: 0.02 $N\text{-}NO_2$: 0	TN: 41.7	TN: 29.2 $N\text{-}NO_3$: 6.91 $N\text{-}NO_2$: 16.8	–	–	N: 58.3	N: 20.0	N: 21.7	Su et al. (2012a)
		Operation: 14 days 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 10:1			TN: 58.6	TN: 20.7 $N\text{-}NO_3$: 4.81 $N\text{-}NO_2$: 13.4	–	–	N: 41.4	N: 24.9	N: 33.7	
		Operation: 14 days 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 5:1			TN: 91.0	TN: 4.51 $N\text{-}NO_3$: 2.3 $N\text{-}NO_2$: 0.0	–	–	N: 9.0	N: 60.0	N: 31.0	
		Operation: 14 days 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 1:1			TN: 86.0	TN: 7.01 $N\text{-}NO_3$: 2.0 $N\text{-}NO_2$: 1.9	–	–	N: 14.0	N: 41.6	N: 44.4	
		Operation: 14 days; 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 1:5			TN: 50.2	TN: 24.9 $N\text{-}NO_3$: 1.6 $N\text{-}NO_2$: 20.7	–	–	N: 49.8	N: 14.9	N: 35.3	
		Operation: 14 days 7000 lux; L:D = 12:12; 13 °C Algae:sludge ratio = 0:1			TN: 18.6	TN: 40.8 $N\text{-}NO_3$: 6.46 $N\text{-}NO_2$: 0.0	–	–	N: 81.4	N: 11.7	N: 6.9	
Revolving Algal Biofilm 1000 L Height:0.9 m	Algal consortium from the clarifiers of a WWTP	Semi-continuous; Operated: 180 days HRT: 1.3 days; 10–30 °C	Pre-treatment effluent	TP: 2–11[a]	TP: 44[a]	TP: 1.12–6.16	26[a]	P: 2.3[a]	P: 56	P: 5.4–29.9	P: 14.1–38.6	Zhao et al. (2018)
		Semi-continuous; Operated: 180 days HRT: 4.7 days; 10–30 °C			TP: 62[a]	TP: 0.76–4.18	19.5[a]	P: 1.7[a]	P: 38	P: 3.0–16.6	P: 45.4–59	
		Semi-continuous; Operated: 180 days HRT: 7 days; 10–30 °C			TP: 56[a]	TP: 0.88–4.84	6.5[a]	P: 1.4[a]	P: 44	P: 0.8–4.6	P: 51.5–55.2	
Revolving Algal Biofilm 1000 L Height:1.8 m		Semi-continuous; Operated: 180 days HRT: 1.3 days; 10–30 °C			TP: 64[a]	TP: 0.72–3.96	45.5[a]	P: 2.2[a]	P: 36	P: 9.1–50.1	P: 14–54.9	
		Semi-continuous; Operated: 180 days HRT: 4.7 days; 10–30 °C			TP: 90[a]	TP: 0.2–1.1	28.6[a]	P: 1.3[a]	P: 10	P: 3.4–18.6	P: 71.4–86.6	
		Semi-continuous; Operated: 180 days HRT: 7 days; 10–30 °C			TP: 90[a]	TP: 0.2–1.1	11.7[a]	P: 1.3[a]	P: 10	P: 1.4–7.6	P: 82.4–88.6	

[a] Approximate values read from graphs.

The mass balance results on these studies show the importance of removal mechanisms other than microalgae uptake for the reduction of the C, *N* and P contents of the wastewaters. Therefore, the often-reported assumption that the main removal mechanism is microalgae uptake may not always be correct, and requires substantiation. Aiming at carbon and nutrient recovery, process optimization must be guided by more accurate measurements not only on biomass productivity, but also on the extent of losses through the release to the atmosphere.

The studies performed by Posadas et al. (2013) and Su et al. (2012a) also show the importance of the nitrification process during the microalgae cultivation step. The conversion of ammonium to nitrate and nitrite may affect nitrogen removal efficiency though microalgae uptake, depending of the species' nutritional adaptations. The quantification of nitrate and nitrite is also essential to guide subsequent process options on the fate of this nutrient, whether through the promotion of denitrification and associated *N* loss into the atmosphere or through the use of the treated water for irrigation. The nitrogen and phosphorus contents in the used cultivation effluents were variable, resulting in different N/P mass ratios with values between 2 and 19 (see Table 9). Depending on the microalgae strain, namely its elemental composition ranges and ability to accumulate storage compounds (e.g., lipids), the ideal nutrient ratio for the process can vary and even involve growth limitation when one of the essential nutrients is depleted. Using a microalgae cultivation step for wastewater treatment leads to the production of additional biomass, thus intentionally retaining a larger

fraction of the input carbon and nutrient loads, including fractions previously mineralized by bacterial (e.g., activated sludge) action. Microalgae biomass can have various applications, some with high added value, but in this review's context these are markedly limited by the risks (mainly health related) associated to the source of the growth media, domestic wastewater. Anaerobic digestion is one of the possible valorization routes for microalgae biomass cultivated on wastewater, as proposed in Fig. 1. The possibility of increasing the methane production in the sewage sludge anaerobic stabilization step (Caporgno et al., 2015), would be an important part of an overall energy balance to microalgae systems, which is a circular economy requirement in addition to the elemental mass balances. This aspect is however not covered in any of the studies of Table 9.

Anaerobic digestion is a wellknown process typically used in WWTP for the stabilization of the sludge produced during the wastewater treatment process (Caporgno et al., 2015; Chen et al., 2018; Kannah et al., 2021; Passos et al., 2015). During this process, the sludge is converted to biogas, a valuable biofuel mostly composed of methane and CO_2, and a digestate. The latter is further processed to produce a dewatered, stabilized sludge and an aqueous centrate which is typically recycled to the beginning of the wastewater treatment process or used as a fertilizer in agriculture (Caporgno et al., 2015; Jankowska et al., 2017; Saratale et al., 2018).

The anaerobic co-digestion of microalgae with the sludge produced in other steps of the WWTP is one of the simplest and most widespread applications for microalgae biomass produced in wastewater, presenting least obstacles to be overcome (González-Fernández et al., 2016). In fact, depending on the conditions, the co-digestion can improve the overall conversion of carbon to methane, when compared to the digestion of the separate feeds, therefore increasing the energy yield (Arias et al., 2018; Saratale et al., 2018). Also, there is the possibility to recycle CO_2 from the upgrading of the produced biogas to the microalgae cultivation system, reducing the carbon emissions, and enhancing the microalgae growth, which in turn leads to increased methane production (Chen et al., 2018; Jankowska et al., 2017; Saratale et al., 2018). Moreover, microalgae cultivation conditions, e.g. under nitrogen starvation, which favours lipid accumulation have been shown to improve the biomethane potential of the resulting biomass even when compared to sewage sludge (Caporgno et al., 2015), presumably also helping to mitigate the risk of ammonia accumulation in the anaerobic digester.

A major difficulty related to microalgae biomass digestion is the typical requirement of a pre-treatment process to break the complex cell wall (Jankowska et al., 2017; Kannah et al., 2021; Passos et al., 2015). Also, different microalgae species present distinct morphological and biochemical characteristics which makes the choosing of a pre-treatment process species specific, i.e., there is no single ideal process for all microalgae biomass types (González-Fernández et al., 2016; Passos et al., 2015). Thus, the study of the microalgae consortium is valuable to determine the best operation conditions and the optimal pre-treatment process that allows a more efficient digestion. Some reviews have been published on the studied and typically employed pre-treatment methods (Jankowska et al., 2017; Kannah et al., 2021; Saratale et al., 2018), namely mechanical, thermal, biological and chemical.

4 Conclusions

Of the many studies which have been performed on wastewater treatment using microalgae cultivation, a poll of 119 recently published reports was here examined, focusing on the collection of data on carbon and nutrient recovery and comprising a wide range of feed wastewater types diverted from different locations in the classical WWTP process.

Depending on the feed type and conditions tested, high removal efficiencies were achieved for nitrogen, phosphorus, and carbon, confirming that this is a promising option towards a circular economy in the wastewater treatment sector. However, few studies were found which monitored the fate of organic and inorganic carbon and few reported on full scale operations. This circumstance confirms the knowledge gap which the present review proposed to assess, namely the scarcity of experimental data enabling full mass balances to the main nutrients, C, *N* and P.

Since the main intended recovery route for these valuable elements is the cultivated biomass, elemental analysis on it needs to be performed. This would quantify the actual fraction of removal which occurs through microalgae uptake, helping to direct control actions aiming to minimize other processes (e.g., phosphorus precipitation, ammonia volatilization). Only a few studies disclosed data on this elemental analysis, actually confirming the importance of other removal mechanisms. In some cases, assumptions on the basis of the operational conditions could be made (e.g., insignificant ammonia volatilization at the lower pH range). Nine of the published reports disclosed enough detailed data enabling overall mass balance calculations on C, *N* and P. These tentative mass balances again confirmed that removal routes other than microalgae uptake can play major roles and impair carbon and nutrient recycling through the biomass. Thus, a focus on mass balances would help in the correct assessment of operational adjustments towards directing more of the recoverable elements to biomass growth. For this, future experiments have to intensify their analytical efforts in order to cover as many of the system outputs as possible, namely the dissolved and suspended/sedimented forms of the target elements, since the gaseous outputs are difficult to measure in open systems. Medium to large scale experiments over prolonged time frames, in order to cover influent and seasonal parameter variations are also scarce.

In terms of microalgae biomass applications in this context of wastewater treatment, anaerobic digestion is showing to be a promising option. The process is already implemented in current WWTPs, being a well-known mature technology and representing reduced investment costs in comparison with other uses of the harvested microalgae. Moreover, co-digestion of the latter with WWTP sludge can lead to an improvement of the biogas production efficiency and does not entail the health-related obstacles imposed by other uses on microalgae cultivated on wastewater (e.g., food and feed,

cosmetics or pharmaceuticals). Finally, sludge and biomass stabilization through anaerobic digestion is the main route for resource recovery, through both bioenergy and biofertilizer products, together with reusable clean water, in the circular economy concept for wastewater treatment. The possible roles of microalgae cultivation and co-digestion in enhancing the productivity and quality of these outputs is worthy of assessment in the near future. For this, experimental data on energy inputs and outputs needs to be produced, enabling energy balances together with the elemental mass balances.

CRediT authorship contribution statement

Sofia A. Vaz: Investigation, Writing – original draft, Writing – editing. **Sara M. Badenes:** Conceptualization, Investigation, Writing – review & editing, Project administration. **Helena M. Pinheiro:** Conceptualization, Writing – review & editing, Project administration. **Rui C. Martins:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

See Table A.1.

Table A.1
Published results for microalgae cultivation on wastewater performed in lab scale vessels.

Reactor	Inoculum	Operation conditions	Wastewater type	Influent pollutant load (mg/L)	Pollutant removal yield (%)	Biomass production (dry weight)	Ref.
Roux bottle (1 L)	*Chlorella kessleri* (Ck) *Chlorella prototthecoides* (Cp)	Batch Operation: 11 days 30–200 μmol m^{-2} s^{-1} L:D = 4:20 | 24:0 27 °C Air and air+5% CO_2	Centrate (filtered and autoclaved)	–	Ck: COD: 74.89–89.96 TP: 12.44–92.39 TN: 61.12–83.94 $N–NH_4$: 100 Cp: COD: 80.78–87.77 TP: 13.17–82.54 TN: 84.43–86.74 $N–NH_4$: 100	Ck: 2.01 g/L Cp: 1.31 g/L	Li et al. (2011b)
Pyrex Roux bottle (1 L)	*Auxenochlorella protothecoides* UMN280	Batch Operation: 12 days Air | air+1% CO_2 | air+5% CO_2 60 μmol m^{-2} s^{-1} pH: 6.5–9.5 Mixotrophic mode	Centrate (autoclaved)	COD: 2324 $P–PO_4$: 212 $N–NH_3$: 91 TN: 134 TOC: 970 TKN: 134 $N–NO_2$: <0.03 $N–NO_3$: 0.35	Air: COD: 81.40 $P–PO_4$: 75.05 $N–NH_3$: 72.97 TN: 73.63 Air+CO_2 (1% or 5%): COD: 80.13 (1) | 78.91 (5) $P–PO_4$: 26.90 (1) | 25.40 (5) $N–NH_3$: 52.06 (1) | 69.31 (5) TN: 64.51 (1) | 68.78 (5)	Air: 0.975 g/L Air+1% CO_2: 2.01 g/L Air+5% CO_2: 2.51 g/L	Zhou et al. (2012b)
Erlenmeyer flask (250 mL)	*Auxenochlorella protothecoides* UMN280	Batch Operation: 12 days L:D = 0:24 pH: 6.2–9.5 Air | air+1% CO_2 | air+5% CO_2 60 μmol m^{-2} s^{-1} Heterotrophic mode	Centrate (autoclaved)	COD: 2324 $P–PO_4$: 212 $N–NH_3$: 91 TN: 134 TOC: 970 TKN: 134 $N–NO_2$: <0.03 $N–NO_3$: 0.35	Air: COD: 79.10 $P–PO_4$: 91.00 $N–NH_3$: 100 TN: 90.22 Air+CO_2 (1% or 5%): COD: 74.40 (1) | 70.56 (5) $P–PO_4$: 98.48 (1) | 98.34 (5) $N–NH_3$: 100 (1) | 100 (5) TN: 90.60 (1) | 89.55 (5)	Air: 1.81 g/L Air+1% CO_2: 2.23 g/L Air+5% CO_2: 2.28 g/L	Zhou et al. (2012b)
Erlenmeyer flask (250 mL)	*Haematococcus pluvialis*	Operation: 18 days 50 μmol m^{-2} s^{-1} | 200 (induction); L:D = 12:12 23 °C ; Air+5% CO_2	Primary ef. (filtered)	$N–NH_4$: 0.6 $N–NO_3$: 42.4 $P–PO_4$: 2.6 COD: 22.0	$N–NO_3$: 100 $P–PO_4$: 100	0.24–0.78 g/L (before and after induction)	Kang et al. (2006)

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Table A.1 (*continued*).

Borosilicate bioreactor (2 L)	*Chlorella vulgaris*	Batch Operation: 12 days 150 μmol m^{-2} s^{-1}; L:D = 14:10 20 °C ; Air; pH: 7.1–8.1	Pre-treatment, primary, secondary, disposing ef. and centrate	Pre-treatment ef.: TP: 6.07 | 5.93 $P–PO4^{3-}$: 4.93 | 4.89 TN: 84.42 | 41.96 $N–NH_4^+$: 80.5|39.55 $N–NO_3^-$: 2.94 | <0.5 $N–NO_2^-$: 0.18 | 0.02 COD: 150 | 180 Primary | secondary ef.: TP: 3.20 | 7.51 $P–PO_4^{3-}$: 1.7 | 7.39 TN: 33.9 | 65.65 $N–NH_4^+$: 30.6|48.79 $N–NO_3^-$: <0.5 | <0.5 $N–NO_2^-$: <0.02| 0.02 COD: 160 | 90 Disposing ef.: TP: 2.38 | 0.76 $P–PO_4^{3-}$: 2.12 | 0.68 TN: 36.44 | 10.3 $N–NH_4^+$: 23.34|4.06 $N–NO_3^-$: 7.23 | 7.03 $N–NO_2^-$: 1.26 | 0.27 COD: 90 | 100 Centrate: TP: 55.01 $P–PO_4^{3-}$: 35.3 TN: 123.9 $N–NH_4^+$: 125.1 $N–NO_3^-$: <0.5 $N–NO_2^-$: <0.02 COD: 675	Pre-treatment ef.: TP: 100 | 100 TN: 94 | 100 COD: 40 | 42 Primary | secondary ef.: TP: 100 | 100 TN: 98 | 95 COD: 44 | 50 Disposing ef.: TP: 100 | 79 TN: 53 | 85 COD: 33 | 25 Centrate: TP: 25 TN: 95 COD: 56	Pre-treated ef: 115.7 | 117.0 mg L^{-1} d^{-1} Primary ef.: 55.65 mg L^{-1} d^{-1} Secondary ef.: 128.2 mg L^{-1} d^{-1} Disposing effluent: 79.82 | 73.88 mg L^{-1} d^{-1} Centrate: 195.1 mg L^{-1} d^{-1}	Cabanelas et al. (2013b)
Cylinder flask (500 mL)	*Neochloris oleoabundans*	Batch Operation: 7 days 1280 Lumen 25–30 °C; Air+5% CO_2	Secondary ef. (filtered and enriched with NO_3)	$N–NH_4$: 12.3 $N–NO_3$: 10 COD: 340–560 $P–PO_4$: 3–6	–	233.3 mg L^{-1} d^{-1} NO_3: 2.1 g/L No NO_3: 0.68 g/L	Wang and Lan (2011)
Duran bottle (1 L)	Algal-bacterial consortia (*Coelastrum microporum* IFA9)	Batch Operation: 12 days 120 μmol m^{-2} s^{-1}; L:D = 12:12 | 36:12 | 60:12 20 °C ; Air; pH: 7.3–8.5	From anaerobic digester treating primary and secondary ef.	COD: 178.4 TDN: 39.5 TDP: 5.3	COD: 59–80 TDN: 35–88 TDP: 43–89	0.2–0.65 g/L	Lee et al. (2015)
Flask (200 mL)	*Chlorella vulgaris* (Cv) and *Scenedesmus quadricauda* (Sq)	Operation: 15 days 27 °C ; pH: 7.0–8.4	Domestic	–	COD: 80.6 (Cv) | 71.0 (Sq) BOD: 70.9 (Cv) | 89.2 (Sq) $N–NO_3$: 78.1 (Cv) |70.3 (Sq) $P–PO_4$: 62.7 (Cv) | 81.3 (Sq)	–	Kshirsagar (2013)
Flask (2.5 L)	*Spirulina platensis*	Operation: 8 days 3300–3400 lx; L:D = 12:12 25 °C ; Air; pH: 7.5–9.5	Synthetic municipal wastewater	TN: 25.30 TP: 2.67	TN: 81.51 TP: 80.52	262.50 mg/L	Zhai et al. (2017)
Serum bottles (500 mL)	*Chlorella vulgaris* (Cv) *Scenedesmus obliquus* (So) *Ourococcus multisporus* (Om)	Batch Operation: 7 days 45– 50 μmol photon m^{-2} s^{-1} L:D = 16:8; 27 °C Air+15% CO_2	Tertiary ef. (filtered)	TN: 8,7 TP: 1.71 TC: 22.6 TIC: 14.6 TOC: 8.1 $N–NH_4^+$: 0.4 $N–NO_3^-$: 8.5 $N–NO_2^-$:0	TN: 100 TP: 100	Om: 0.203 g/L Cv: 0.197 g/L So: 0.197 g/L	Ji et al. (2013)
Flask (2 L)	*Chlorella vulgaris*	Batch Operation: 7 days 2000–10000 μmol m^{-2} s^{-1} Air	Mixture of primary (25%) and secondary (75%) ef. (filtered)	Primary ef.: $N–NH_4$: 43.31 $N–NO_3$: 56.19 COD: 256 P–PO4–3: 0.63 Secondary ef.: $N–NH_4$: 0.63 $N–NO_3$: 224.78 COD: 96 $P–PO_4^{-3}$: 0.53	$N–NH_4$: 100 $N–NO_3$: 82 COD: 100	138.76 mg L^{-1} d^{-1}	Ebrahimian et al. (2014)
Erlenmeyer flask (1 L)	*Chlorella sorokiniana*	Operation: 14 days 25 °C Mixotrophic (m): 120 μmol photons m^{-2} s^{-1}; L:D = 16:8 Heterotrophic (h): Dark conditions	Pre-treatment ef. and centrate	Pre-treatment ef.: COD: 474.36 $N–NH_4$: 50.49 $N–NO_3$: 26.00 $N–NO_2$: 120.00 $P–PO_4$: 19.7 Centrate: COD: 185.53 $N–NH_4$: 35.00 $N–NO_3$: 10.70 $N–NO_2$: 70.00 $P–PO_4$: 24.00	Pre-treatment ef. (m or h): COD: 36.68 (m) | 59.15 (h) $N–NH_4$: 89.13 (m) | 27.91 (h) $N–NO_3$: 19.23 (m) | 56.25 (h) $N–NO_2$: 75.00 (m) | 33.33 (h) $P–PO_4$: 87.82 (m) | 13.27 (h) Centrate (mix or het): COD: 44.03 (m) | 59.42 (h) $N–NH_4$: 94.29 (m) | 23.53 (h) $N–NO_3$: 28.67 (m) | 25.00 (h) $N–NO_2$: 57.14 (m) | 25.00 (h) $P–PO_4$: 83.3 (m) | 83.81 (h)	Pre-treatment ef. (m): 72.5 mg L^{-1} d^{-1} 1015 mg/L (h): 61.88 mg L^{-1} d^{-1} 495 mg/L Centrate (m): 77.14 mg L^{-1} d^{-1} 1080 mg/L (h): 76.25 mg L^{-1} d^{-1} 610 mg/L	Ramsundar et al. (2017)

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Table A.1 (*continued*).

Flasks (100 mL)	*Auxenochlorella protothecoides* UMN280	Batch Operation: 6 days	Concentrated municipal wastewater (filtered and autoclaved)	$P–PO_4$: 211 TKN: 134 COD: 2344 TOC: 970	$P–PO_4$: 81.52 TKN: 59.70 COD: 88.99 TOC: 96.18	269 mg L^{-1} d^{-1}	Zhou et al. (2012a)
Conical flask (500 mL)	*Chlorella pyrenoidosa* (Cp) and *Scenedesmus abundans* (Sa)	Batch Operation: 13 days 1800 lux; L:D = 24:0	Influent (autoclaved and not)	$N–NH_3$: 992 $P–PO_4$: 286 $N–NO_3$: 197 chloride (Cl^-): 268 COD: 286 BOD: 236	Cp: $N–NH_3$: 99 (NA) | 99 (A) $P–PO_4$: 96–97 (NA) | 86 (A) $N–NO_3$: 71–78 (NA) | 80 (A) Cl^-: 86–91 (NA) | 88 (A) Sa: $N–NH_3$: 95–99 (NA) | 92 (A) $P–PO_4$: 96–97 (NA) | 96 (A) $N–NO_3$: 74–81 (NA) | 83 (A) Cl^-: 85–91 (NA) | 90 (A)	–	Lekshmi et al. (2015)
Duran bottle (1 L)	*Chlorella sorokiniana* UTEX123	Batch Operation: 4 days 80 μmol m^{-2} s^{-1} 30 °C ; Air+CO_2 (exhaust gas)	Secondary ef. and centrate (autoclaved and diluted 1:10)	Secondary ef.: TN: 8 TP: 2.6 TOC: 2.1 Centrate: TN: 53 TP: 9.4 TOC: 9.56	Secondary ef.: TN: 100 $P–PO_4$: close to 0 Centrate: TN: 100 $P–PO_4$: 40	Secondary ef.: 220–250 mg/L Centrate: 170–330 mg/L	Lizzul et al. (2014)
Erlenmeyer flask (500 mL)	*Chlorella*	Batch Operation: 11 days 4000 lux; L:D = 12:12 26 °C ; Flue gas	Kitchen and sewage wastewater	Kitchen: $N–NO_3$: 52.962 $P–PO_4$: 2.037 COD: 560 BOD: 65 N–NH4: 30 Sewage: $N–NO_3$: 30.25 $P–PO_4$: 1.7 COD: 784 BOD: 80 $N–NH_4$: 22	Kitchen: $N–NO_3$: 38 $P–PO_4$: 75 COD: 32 Sewage: $N–NO_3$: 67 $P–PO_4$: 88 COD: 75	Kitchen: 0.6 g/L Sewage: 0.45 g/L	Kumar et al. (2019)
Pyrex bottles (5 L)	*Kirchneriella* sp.	Batch Operation: 14 days 90 μmol m^{-2} s^{-1}; L:D=20:4; 20 °C ; Air+1% CO_2	Municipal	TKN: 2.9 TP: 17.0	–	0.6 g/L	Frampton et al. (2013)
Glass bottle (250 mL)	*Chlorella protothecoides*	Batch Operation: 5–10 days 100 μE m^{-2} s^{-1}; L:D = 24:0 23 °C ; Air+5% CO_2	Primary and secondary ef.	Primary ef.: $N–NO_3$: 9.31–18.83 $N–NH_4$: 44.46–63.83 P: 8.00–9.44 COD: 34–130 Secondary ef.: $N–NO_3$: 24.67–34.67 $N–NH_4$: 0.89–1.66 P: 1.02–1.64 COD: 0–34	Primary ef.: $N–NO_3$: >90 $N–NH_4$: 94–97 P: 62–70 COD: 0 Secondary ef.: $N–NO_3$: >90 $N–NH_4$: 4–33 P: 50–68 COD: 0	–	Tercero et al. (2014)
Conical flasks (500 mL)	*Micractinium reisseri*	Operation: 8 days 40 μmol photon m^{-2} s^{-1}; L:D = 24:0; 27 °C	Influent, secondary and tertiary ef. (filtered)	Influent: TN: 15 TP: 3 TIC: 6.7 Secondary ef.: TN: 13 TP: 2 TIC: 4.4 Tertiary ef.: TN: 11 TP: 1.6 TIC: 3.5	Influent: TN: 86 TP: 95 Secondary ef.: TN: 85 TP: 96 Tertiary ef.: TN: 89 TP: 95	Influent: 0.22 g/L Secondary effluent: 0.19 g/L Tertiary effluent: 0.14 g/L	Abou-Shanab et al. (2014)
Erlenmeyer flask (500 mL)	*Chlorella vulgaris*	Batch and continuous Operation: 17 days 54 μmol m^{-2} s^{-1}; L:D = 12:12 26 °C Not aerated (het) | flue gas (mix)	Sewage wastewater (filtered)	COD: 520 $N–NO_3$: 3.840 $P–PO_4$: 1.189 BOD: 80	Batch (het or mix): COD: 78 (het) | 75 (mix) $N–NO_3$: 87 (het) | 95 (mix) $P–PO_4$: 67 (het) | 49 (mix) Continuous: COD: 42 (het) | 40 (mix) $N–NO_3$: 83 (het) | 91 (mix) $P–PO_4$: 52 (het) | 55 (mix)	Batch: 0.4 g/L at day 12 and then decreases (het) 0.6 g/L (mix) Continuous: 0.6 g/L steady (het) 1.0 g/L (mix)	Kumar et al. (2018)
Erlenmeyer flask (500 mL)	*Scenedesmus* sp. ZTY1	Operation: 21 days 50– 60 μmol photon m^{-2} s^{-1}; L:D = 14:10; 25 °C	Primary and secondary ef.	Primary ef.: DOC: 112 TN: 41 TP: 8.4 COD: 235 $N–NH_4^+$: 32.7 Secondary ef.: DOC: 28 TN: 11 TP: 1.9 COD: 41 $N–NH_4^+$: 0.14	Primary ef.: DOC: 72.1 TN: 92.9 TP: 99.2 Secondary ef.: DOC: 62.1 TN: 90.0 TP: 97.4	Primary ef.: 3.8×10^6 cells/mL Secondary ef.: 1.9×10^6 cells/mL Maximum dry weight: 478 mg/L	Zhang et al. (2014)

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Table A.1 (*continued*).

Glass beaker (5 L)	*Phormidium* sp. (Ps), *Chlamydomonas reinhardtii* (Cr), *Chlorella. Vulgaris* (Cv) and *Scenedesmus rubescens* (Sr)	Batch Operation: 7 days 7000 lux; L:D = 12:12	Secondary ef.	$N–NH_4$: 25.2 $P–PO_4$: 1.74 $N–NO_3$: 0.75 COD: 30.2 TKN: 26.4 $N–NO_2^-$: 0.10	$N–NH_4$: 100 $P–PO_4$: 100 $N–NO_3$: >93	Ps: 2.71 g m^{-2} d^{-1} tabbr Cr: 6.06 g m^{-2} d^{-1} Cv: 6.28 g m^{-2} d^{-1} Sr: 6.56 g m^{-2} d^{-1}	Su et al. (2012b)
Plastic and glass bottles (5 L)	*Chlorella variabilis* TH03	Batch Operation: 14 days 15 μmol m^{-2} s^{-1}; L:D = 16:8; 19.4–23.9 °C Air; pH: 8.9–9.8	Discharge ef. (autoclaved and not)	COD: 124.45–168.16 $N–NH_4$: 65.74–71.9 TP: 4.75–5.73 $N–NO_3$: 0.059–0.11 $N–NO_2$: 0.283–0.14 $P–PO_4$: 3.83–4.29 TN: 69.61–72.24	COD: 79.3 (NA) | 74.8 (A) $N–NH_4$: 96.3 (NA) | 93.3 (A) TP: >97.1 (NA) | >97.1 (A)	1.52 g/L (NA) 1.67 g/L (A)	Tran et al. (2021)
Conical flask (2 L)	*Oscillatoria tenuis*	Batch Operation: 7 days 350 mol m^{-2} s^{-1}; L:D = 24:0 25 °C ; Air; pH: 7.5–10.2	Secondary ef.	$N–NH_4$: 10.2 TP: 0.8 COD: 112.8	$N–NH_4$: 92.1–96.1 TP: 75.4–82.9 COD: 88.4–92.6	104–150 mg L^{-1} d^{-1}	Cheng et al. (2018)
Erlenmeyer flask (500 mL)	*Chlorella* sp.	Batch Operation: 24 days 60 μmol photon m^{-2} s^{-1}; L:D = 12:12; 25 °C	Influent and secondary ef. (filtered and autoclaved)	Influent: TN: 38.01 TP: 1.83 N–NOx: 0.75 $N–NH_4$: 16.30 $P–PO_4$: 0.64 Secondary ef.: TN: 29.45 TP: 0.57 N–NOx: 24.4 $N–NH_4$: 1.13 $P–PO_4$: 0.52	Influent: TN: 58.85 TP: 97.08 Secondary ef.: TN: 42.93 TP: 91.98	–	Wang et al. (2013a)
Shake flask (250 mL)	*Chlorella vulgaris*	Batch Operation: 21 days L:D = 12:12	Municipal	–	COD: 66 BOD: 70 TP: 67 TN: 71 DS: 51 VS: 54	–	Sahu (2014)
Erlenmeyer flask (1 L)	*Chlorella pyrenoidosa*	Operation: 6 days 80 μmol m^{-2} s^{-1}; L:D = 12:12 Air	Synthetic wastewater	COD: 5000 $N–NO_3$: 100 $P–PO_4$: 40 Dilutions: 5000 | 3000 | 1000 mg/L COD	COD: 43 (5000) | 61–66 (1000 | 3000) $N–NO_3$: 70–99 $P–PO_4$: 80–99 TOC: 90 (1000) | 65 (3000) | 35 (5000)	–	Gupta et al. (2017)
-	Two strains of *Chlorella sorokiniana* (A) | (B)	Batch Operation: 36 (A) | 30 (B) h 90 μmol m^{-2} s^{-1}; L:D = 12:12 25 °C	Secondary ef.	$N–NH_4$: 3.4 $N–NO_3$: 2.5 TKN: 5.9 $P–PO_4$: 3.4 BOD: 35 COD: 250	TN: 80 (A) | 18 (B) $N–NO_3$: 72 (A) | 53 (B) TKN: 91 (A) | 12 (B) $P–PO_4$: 80 (A) | 92 (B) BOD: 61 (A) | 57 (B) COD: 37 (A) | 27 (B)	124 (A) | 78 (B) mg L^{-1} d^{-1}	Rani et al. (2020)
Flask	*Scenedesmus* sp. ISTGA	Batch Operation: 14 days L:D = 18:6; pH: 6.3–8.2	Influent	BOD: 190.1 COD: 456.3 $N–NO_3$: 15.3 $P–PO_4$: 9.8	BOD: 86.43 COD: 88.82 $N–NO_3$: 100 $P–PO_4$: 100	0.81 g/L	Tripathi et al. (2019)
Erlenmeyer flask (1 L)	*Scenedesmus obliquus*	Operation: 7 days 100 μmol photons m^{-2} s^{-1}; L:D = 12:12; 25 °C	Primary ef.	COD: 286.78 TN: 34.47 $N–NH_4$: 20.68 TP: 2.53	COD: 85.43 TN: 80.30 $N–NH_4$: 87.25 TP: 95.72	0.583 g/L	Qu et al. (2020)
Erlenmeyer flask (500 mL)	*Botryococcus* sp. NJD-1 (Bs), *Scenedesmus* sp. NJD-2 (Ss2), *Chlorella* sp. NJD-3 (Cs) and *Scenedesmus* sp. NJD-5 (Ss5)	Operation: 10 days Dark conditions 30 °C ; pH: 7	Primary ef. (autoclaved and not)	Autoclaved: TOC: 77.97 $N–NO_3$: 15.46 $P–PO_4$: 2.57 Not autoclaved: TOC: 78.97 $N–NO_3$: 15.77 $P–PO_4$: 2.65	Bs: $N–NO_3$: 64.5 (NA) | 41.1 (A) $P–PO_4$: 89.8 (NA) | 89.5 (A) TOC: 67.9 (NA) | 75.5 (A) Ss2: $N–NO_3$: 52.2 (NA) | 46.3 (A) $P–PO_4$: 88.5 (NA) | 87.8 (A) TOC: 70.8 (NA) | 88.5 (A) Cs: $N–NO_3$: 50.1 (NA) | 37.1 (A) $P–PO_4$: 77.4 (NA) | 79.1 (A) TOC: 65.1 (NA) | 99.1 (A) Ss5: $N–NO_3$: 51.1 (NA) | 45.5 (A) $P–PO_4$: 87.7 (NA) | 96.1 (A) TOC: 73.9 (NA) | 99.9 (A)	Bs: 1.17 g/L (NA) 0.93 g/L (A) Ss2: 2.02 g/L (NA) 1.19 g/L (A) Cs: 1.61 g/L (NA) 0.76 g/L (A) Ss5: 0.54 g/L (NA) 0.43 g/L (A)	Shen et al. (2017)
Erlenmeyer flask (250 mL)	*Auxenochlorella protothecoides* UMN280	Batch Operation: 7 days L:D = 0:24; pH: 6.2–9.5	Centrate (autoclaved)	COD: 2324 $P–PO_4$: 212 $N–NH_3$: 91 TN: 134	COD: 88.99 TP: 81.52 $N–NH_3$: 69.23 TN: 59.70	1.12 g/L	Zhou et al. (2012b)
Erlenmeyer flask (1 L)	*Chlorella* sp. 227	Batch Operation: 9 days 60 μmol m^{-2} s^{-1}; L:D = 24:0 25 °C	Secondary ef. (filtered and UV radiation)	TN: 18.9 TP: 1.7 BOD: 6.9 COD: 11.2 $N–NH_4$: 10.0 $N–NO_3$: 6.6	TN: 92 TP: 86	0.024–0.074 g L^{-1} d^{-1}	Cho et al. (2011)

(*continued on next page*)

Table A.1 (*continued*).

Glass tubes (3 × 500 mL)	*Scenedesmus obliquus*	Batch Operation: 6 days 26 °C; Air	Primary and secondary ef.	Primary ef.: $N–NH_4$: 12.72–40.55 $P–PO_4$: 1.12–2.69 Secondary ef.: $N–NH_4$: 4.23–8.85 $P–PO_4$: 0.44–1.74	$N–NH_4$: 99 $P–PO_4$: 99	Primary ef.: 2.5–3.0 g/L Secondary ef.: 2.2–2.5 g/L	Ling et al. (2019)
Beaker (1 L)	Consortia of native filamentous microalgal strains	Batch Operation: 10 days 420–1760 μmol photons m^{-2} s^{-1} 17–36 °C ; pH: 8.2–10.2	Primary ef.	$N–NO_3$: 83.714 $N–NH_4$: 21.067 $P–PO_4$: 3.147 BOD: 2.4 COD: 2150	$N–NO_3$: 90 $N–NH_4$: 100 $P–PO_4$: 97.8 BOD: 99 COD: 87	1.07 g/L	Renuka et al. (2013)
Pyrex flask (2 L)	*Chlorella vulgaris* (Cv), *Chlorella kessleri* (Ck), *Scencedesmus obliquus* (Sc) and a natural bloom (BL)	Batch Operation: 10 days 250 μmol m^{-2} s^{-1}; L:D = 14:10 20 °C ; Air+5% CO_2	Secondary effluent	TN: 22.6 $P–PO_4$: 5.6	TN: >90 $P–PO_4$: >98	Cv: 112.5 mg L^{-1} d^{-1} Ck: 111.3 mg L^{-1} d^{-1} Sc: 152.3 mg L^{-1} d^{-1} Bl: 167.3 mg L^{-1} d^{-1}	Arbib et al. (2014)
Ilmabor bottles (1 L)	*Desmodesmus communis* + consortium of algae	Batch Operation: 22 days 88–440 μE m^{-2} s^{-1}; L:D = 16:8 28–25 °C ; Air+2% CO_2	Primary and secondary ef.	Primary ef.: $N–NH_3$: 30.12–33.62 P: 1.54–200 Secondary ef.: $N–NH_3$: 0.24 P: <0.01	$N–NH_3$: >99 P: >99	Primary ef.: 0.018–0.138 g L^{-1} d^{-1} Secondary ef.: 0.023 g L^{-1} d^{-1}	Samorì et al. (2013)
Erlenmeyer flask (250 mL)	*Chlorella* sp.	Batch Operation: 14 days	Centrate	$N–NH_3$: 82.5 TN: 116.1 TP: 212.0 COD: 2304.0	$N–NH_3$: 93.9 TN: 89.1 TP: 80.9 COD: 90.8	0.92 g/L	Li et al. (2011a)
Erlenmeyer flask (500 mL)	Mixed culture *Chlorella vulgaris* *Planktothrix isothrix*	Batch Operation: 9 days 20 | 60 μmol photons m^{-2} s^{-1}; L:D = 24:0; 28 °C ; pH: 7–7.8	Secondary effluent	$N–NH_4$: 79.3 $P–PO_4$: 7.47	$N–NH_4$: 80 $P–PO_4$: 100	25.54– 65.54 mg L^{-1} d^{-1}	Silva-Benavides and Torzillo (2012)
Erlenmeyer flask (1 L)	*Chlorella vulgaris* (Cv) *Botryococcus terribilis* (Bt)	Batch Operation: 13 days (*Cv*) | 18 days (*Bt*) 174 μE m^{-2} s^{-1}; L:D = 12:12 25 °C ; Air+2.5% CO_2	Pre-treatment ef. (addition of glycerol)	COD: 995–6078 TN: 43.67–52.41 $P–PO_4$: 6.66–11.59	COD: >70% Almost complete removal of N and P	*Chlorella*: 118 mg L^{-1} d^{-1} *Botryococcus*: 282 mg L^{-1} d^{-1}	Cabanelas et al. (2013a)
Enclosed jacketed glass bioreactor (1 L)	Inoculum dominated by *Chlorella sorokiniana*	Semi-continuous Operation: 23 days HRT: 3 days 122 μmol m^{-2} s^{-1}; L:D = 14:10 24 °C ; pH: 9.5–11	Primary effluent	sCOD: 290.2 $N–NH_4$: 34.1 $P–PO_4$: 4.9	sCOD: 64.2 $N–NH_4$: 71.4 $P–PO_4$: 68.3	330 mg VSS/L	Barreiro-Vescovo et al. (2021)
Pyrex Roux bottle (1 L)	Mixture of green algae and diatoms	Semi-continuous Operation: 18 days HRT: 2–4 days 4300 lux; L:D = 16:8 23–25 °C ; Air | air+CO_2 pH: 7–8	Primary effluent	$N–NH_4$: 39 $P–PO_4$: 2.1	Air: $N–NH_4$: 84 $P–PO_4$: >99 Air+CO_2: $N–NH_4$: >99–98 $P–PO_4$: >99–93	–	Woertz et al. (2009)
Flask (1 L)	*Nostoc muscorum* (Nm), *Navicula veneta* (Nv), *Chlorella vulgaris* (Cv) and consortium of all three	Batch Operation: 7 days 100 μmol m^{-2} s^{-1} L:D = 16:18; 20 °C pH: 7–8.5; Air	Primary ef. (autoclaved and filtered)	SS: 500 BOD: 400 COD: 600 TN: 60 TP: 6.7	Nv | Cv | Nm | Consortium: TN: 96.9 | 94.0 | 72.0 | 84.2 TP: 99.8 | 98.6 | 88.3 | 86.0 COD: 95.7 | 92.6 | 85.7 | 88.3	Nv | Cv | Nm | Consortium: 0.842 | 0.833 | 0.805 | 0.816 day^{-1}	Sisman-Aydin (2022)
Flask (2.5 L)	*Spirulina platensis* (Sp) and *Scenedesmus obliquus* (So)	Batch Operation: 10 days 2000–4000 (Sp) | 4000–8000 (So) lux L:D = 8:16 | 12:12 | 18:8 25 °C; Air	Domestic	–	Sp | So: TN: 100 | 93.8 TP: 88.6 | >99	Max Sp | So: 334 | 380 mg/L	Fan et al. (2020)

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