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Tertiary urban wastewater treatment with microalgae natural consortia in novel pilot photobioreactors

Etiele Greque de Morais[a], José Carlos Amaro Marques[a], Paulo Ricardo Cerqueira[a], Cláudia Dimas[b], Vânia Serrão Sousa[c], Nuno Gomes[d], Margarida Ribau Teixeira[c], Luís Miguel Nunes[e], João Varela[a,f], Luísa Barreira[a,f,*]

[a] CCMAR—Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal
[b] Águas do Algarve, Rua do Repouso, n° 10, 8000-302, Faro, Portugal
[c] CENSE—Centre for Research on the Environment and Sustainability & CHANGE—Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal
[d] Bluemater Eco-eficient Solutions, 4050-049, Porto, Portugal
[e] CERIS—Civil Engineering Research and Innovation for Sustainability, Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, Faro, 8005-199, Portugal
[f] GreenCoLab—Green Ocean Technologies and Products Collaborative Laboratory, CCMAR, University of Algarve, 8005-139, Faro, Portugal

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ABSTRACT

The aim of this work was to evaluate the efficiency of the new GreenDune photobioreactors for tertiary wastewater treatment, treated wastewater reuse and biomass application, using naturally occurring microalgae consortia. The study was conducted on a pilot installation in a wastewater treatment plant in Portugal and different operational conditions were tested. The system was capable to remove up to 95% of NH_4^+, the main pollutant in wastewater after secondary treatment using hydraulic retention times as low as 24 h. The application of a non-conservative scenario allowed the reuse of treated wastewater for seed production, and irrigation of naturally restricted use areas. The produced biomass was rich in proteins and carbohydrates with potential for biofuel production such as biogas or use as biofertilizers, closing the energy and nutrients cycle. Finally, the life cycle assessment of both the GreenDune and existing nitrification/denitrification systems were compared revealing that the operation of the GreenDune are more environmentally favourable than the existing system.

1. Introduction

Water reuse is a solution for scarcity, reducing hydrological stress and providing a reliable water supply. In Europe, only 2.4% of treated urban wastewaters are reused per year (Wintgens and Hochstrat, 2006). However, wastewater reuse requires more efficient, ecological, economic, and easy-to-manage treatment systems. The improvement and establishment of technologies for wastewater treatment and management, and water conservation and savings, specially using nature-based-solutions, can alleviate future clean water scarcity (Boretti and Rosa, 2019). Recently, Chojnacka et al. (2020) and Mainardis et al. (2022) reviewed fertigation with treated wastewater as a way to use nutrients from wastewaters, in particular nitrogen and phosphorus, in agriculture. The presence of nitrogen, phosphorus, and potassium in wastewaters serves as a fertilizer which are necessary for plant growth.

In most wastewater treatment plants (WWTP), tertiary treatment aims to remove excess phosphorus and nitrogen nutrients, such as phosphate (PO_4^{3-}), nitrates (NO_3^-) and ammonium (NH_4^+), which is essential when treated wastewater discharges into eutrophication-sensitive ecosystems or when reuse is intended (Harrison et al., 2001). The major problems of tertiary treatment are the high energy costs associated with the need for strong aeration and carbon sources, release of greenhouse gases (e.g., carbon dioxide, ammonia, methane, and nitrous oxides resulting from the nitrification/denitrification reactions), and sludge formation (Acién et al., 2016).

Wastewater treatment using microalgae is a sustainable alternative with low greenhouse gases emissions compared to conventional systems and the produced "sludge" is a biomass that could be upgraded for bioproducts contributing to circular economy by re-using and adding value to waste and raw material, including water reuse (Morais et al.,

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[*] Corresponding author. CCMAR—Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.
E-mail address: lbarreir@ualg.pt (L. Barreira).

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2021). Microalgal wastewater treatment can also contribute to increase the reuse of wastewater for fertigation by treating the wastewater to concentrations that allow crop irrigation, abiding EU legislation and minimizing environmental impacts. A very important factor in wastewater treatment with microalgae is the bioreactor design, which needs to promote high biomass productivities in a cost-effective way. Closed photobioreactor (PBR) systems present higher productivities compared to open systems, but they are less used because of their high capital cost and energy usage which would raise dramatically the costs of wastewater treatment (Zittelli et al., 2013). The most used reactors are open raceway ponds, which have low capital and operation costs and are easier to operate. However, they have high land requirements due to the low water column needed for light penetration, which tends to be short, around 15–30 cm (Arbib et al., 2017). For this reason, raceways often present very high volume/area ratios, of 150–300 L m^{-2}. The GreenDune photobioreactors (PBRs) aim to overcome this constraint by a design that allows efficient light penetration and still present a high volume/area ratio, 480 L m^{-2}. These PBRs are open modular systems that can be connected in sequence to increase treatment volume (in case that a higher hydraulic retention time, HRT, is needed for the treatment) and/or in parallel to achieve the total required volume of wastewater to be treated, improving the ease of the scale-up process. This system appeared for the first time on the literature at Barros et al. (2022), where the biomass produced in the system on different seasons was used for biogas production, but no data on wastewater treatment and microalgae growth using the GrennDune PBRs were published.

This new system has some advantages compared to the conventional raceway ponds normally used for wastewater treatment with microalgae: i) the land space (i.e., areal footprint) occupied by this system is around three times lower than conventional raceway systems; and ii) due to its versatility the system is simple to scale up as more modules can be interconnected according to the volume and organic charge of the effluent to be treated, which allows easier system maintenance.

The use of natural consortia for wastewater treatment is another strategy deemed to increase treatment efficiency, as microorganisms naturally present in the wastewater are already adapted to the current environmental conditions, including the chemical composition of the effluent. In these systems microalgae live in close association with other microorganisms forming a mixed consortium of microalgae, bacteria, protozoa, and other organisms. In such a complex system, despite some mixotrophic and heterotrophic microalgae, which may be present in the natural consortia, bacteria are the main responsible for organic carbon removal while releasing carbon dioxide and metabolites, like vitamin B_{12}, that aid microalgal growth (Croft et al., 2005). In turn, microalgae remove inorganic nitrogen and phosphorus nutrients as well as emergent pollutants from the treated water.

The aim of this study was to i) evaluate the efficiency of the newly designed pilot GreenDune PBRs to perform the tertiary treatment of urban wastewater using natural microalgal consortia, ii) evaluate water reuse prospects according to legal parameters, iii) explore possible applications for the produced microalgal biomass, and iv) assess the environmental impact of the proposed solution.

2. Material and methods

2.1. GreenDune photobioreactors pilot system

The novel GreenDune photobioreactors were developed by Bluemater, S.A., a Portuguese company (Fig. 1). The experimental pilot setup presented at Fig. 1 is composed of 3 independent lines (replicates) with 3 GreenDune (GD) module open reactors of 480 L each, which are interconnected at the top and bottom, resulting in a total volume of 1440 L. Each reactor occupies an area of 1 m^2. The reactors are transparent and made of polyacrylate with a prismatic format, maximizing the photic area, and open at the top for gaseous exchange. The system is continuously aerated by porous-curtain aerators located on the front of the system to promote better CO_2 dissolution from air and prevent biofilm formation at the photic area that might hamper light penetration. The system discharges into a settler to collect the produced biomass and discharge clean water. The system was installed at the wastewater treatment plant of Quinta do Lago, in the Algarve, Portugal (37°02′15.9’’N 8°00′32.0’’W). This WWTP performs a preliminary treatment (harrowing, de-sanding, and degreasing) followed by a primary sedimentation (primary treatment), a biological secondary treatment (activated sludge with P precipitation) followed by a secondary sedimentation and a tertiary treatment of nitrification/denitrification process followed by UV disinfection. For this study, the GreenDune photobioreactors received the wastewater after the secondary sedimentation aiming to replace the currently used tertiary treatment (nitrification/denitrification and UV disinfection).

2.2. Microalgae culture conditions

The experiments were conducted in 2020 at outdoor conditions for a period of 1 year covering the 4 different seasons (Table 1). The PBRs were continuously fed with wastewater coming from the secondary settler of the WWTP, so that the tertiary treatment by the GreenDune could be assessed. At least 2 different HRT were tested in each season:

Table 1
Outdoor conditions in each season of the experiments.

Parameter	Winter	Spring	Summer	Autumn
T_{min} (°C)[a]	10.7	17.6	20.0	13.7
T_{max} (°C)[a]	19.5	26.4	29.6	20.0
Precipitation (mm)	1.2	12.0	0	155.8

[a] T_{max} (maximum temperature), T_{min} (minimum temperature).
source: IPMA (2020)

Fig. 1. GreenDune photobioreactors placed at the wastewater treatment plant of Quinta do Lago (Algarve, Portugal).

24h and 48h in winter, summer, and autumn, and 12h and 24h in spring. Two days is a common HRT for wastewater treatment with microalgae (Morais et al., 2021), however, as this study aimed to perform tertiary treatment it was decided to also test a shorter period (24h) and an even shorter one (12h) in spring which is usually the most productive season due to high light abundance and mild temperatures. In each season, the consortium was allowed to form (5–7 days) after which the HRT to be tested was applied and a period of 7 days was given for stabilization of the consortium prior to the beginning of the experiment. Treatment efficiency was followed during the next 12 days. Every 2 days, samples were collected to evaluate biomass concentration inside the PBRs, measured by optical density readings at 750 nm (Biotek, Synergy 4) and converted to dry weight by means of a calibration curve. The pH was evaluated in site in each module using a portable pH meter (Hanna instruments, HI 83141).

2.3. Evaluation of wastewater treatment efficiency

Every two days, the influent and effluent of the GreenDune photobioreactors was evaluated for ammonium (NH_4^+-N), nitrates (NO_3^--N), total nitrogen (NT), phosphates (PO_4^{3-}-P) and chemical oxygen demand (COD) using commercial kits following the manufacturer's instructions. Ammonium was measured with the NANOCOLOR Ammonium 3 tube test (0.04–2.30 mg L^{-1} NH_4–N) and the absorbance measured with a PF-12 Plus photometer (Macherey Nagel, Germany) in filtered samples (0.45 μm). Samples were diluted whenever necessary to fall between the kit's range. Nitrates were measured using the NANOCOLOR Nitrate 250 tube test (4–60 mg L^{-1} NO_3^-N), phosphates with NANOCOLOR ortho and total Phosphate 15 tube test (0.30–15.00 mg L^{-1} P, 1.0–45.0 mg L^{-1} PO_4^{3-}), also in filtered samples. Total N and COD were measured in non-filtered samples using the NANOCOLOR total Nitrogen TNb 22 tube test (0.5–22.0 mg/L N) and NANOCOLOR COD LR 150 Tube test (3–150 mg L^{-1} O_2) after digestion. Total phosphorus (TP) was evaluated by the APHA standard method (APHA method 9221) in non-filtered samples. Total suspended solids (TSS) were determined in the effluent samples by filtration of an adequate amount of sample through cellulose acetate membranes (0.45 μm).

2.4. Wastewater reuse scenarios

The possibility of water reuse was evaluated considering agricultural, urban, and industrial uses using methodology established by EU regulation that defines the minimum requirements for water reuse (2020/741 Regulation (EU), 2020), the Portuguese Law that defines the legal framework to produce water for reuse and its uses (Law decree no. 119/2019, 08/21), Rebelo et al. (2020), and the guidelines defined in Rebelo et al. (2018), since there is no homogeneity between the aspects covered by each Member State regulation. For each physicochemical and microbiological parameters, maximum limit values depend on the intended use (see Tables S1–S3 in Supplementary Information). Salinity is a special parameter that should be measured and controlled when the intend reuse is plant watering (Law decree no. 119/2019). Although its limit depends on crop sensitivity, the Portuguese law recommends a maximum value of 1 dS m^{-1}.

Two scenarios were developed: i) the non-conservative scenario (NCS) that uses the median of the values observed during the analysed period (winter) for each HRT, and ii) the conservative scenario (CS) that considers the maximum of the values observed. The median was used in NCS since it provides a better representation of the most "typical" value. The maximum value was selected for the CS because it represents the worst value obtained for each parameter.

For better assessment of water reuse besides the parameters described in 2.3, treated water collected during the winter season, was also analysed for five-day biological oxygen demand (BOD_5), total suspended solids (TSS), turbidity, and presence of *E. coli*. All parameters were measured using standard methods (Eaton, 2005), except turbidity. Turbidity was estimated by an equation between TSS and turbidity ($r^2 = 0.614$) and was proposed by some authors (Nasrabadi et al., 2016; Rügner et al., 2014).

2.5. Biomass chemical characterization

The produced biomass was harvested from the settlers placed after the PBRs. From each settler, 10 L of concentrated culture were collected and centrifuged (1000×*g* for 10 min, ThermoScientific, Heraeus Megafuge 16R). The biomass was frozen at −20 °C before freeze-drying (Telstar, LyoALFA) for storage until analysis. The dried biomass was evaluated for total lipid content by the gravimetric method of Bligh and Dyer (1959). Carbohydrates and proteins were evaluated after extraction of the intracellular compounds with water aided by bead beating (Retsh, MM 400), for 5 min at 30 Hz. Carbohydrates were determined using the method described by Dubois et al. (1956) using a standard curve with glucose. Total proteins were estimated by the method described by Lowry et al. (1951) using Bovine Serum Albumin (BSA) as standard. Ash and moisture contents were evaluated according to AOAC (Horwitz, 2000) using gravimetric methods.

2.6. Life cycle assessment

The impacts during the construction and operation phases of the technologies were computed following the life cycle assessment (LCA) method (ISO, 2006). The boundaries of the system were set at the entry and exit of the unit operation: the nitrification-denitrification system currently operating at the WWTP, and the GreenDune photobioreactors (more detailed information is provided in Supplementary Material, Fig. S1). Life cycle inventory included all necessary materials for building the unit but excluded ground movement for construction and associated equipment and dismantling of the systems. Flows of energy and materials during the operation phase were quantified from historical data provided by the operator of the WWTP and from the experiments. A lifetime of 25 years was considered for both unit operations.

The calculations were made in OpenLCA® Nexus (version 1.7) (openLCA.org 2019), using Ecoinvent database, v. 3.5 (ECOINVENT 2019). Impacts were quantified using CML Baseline v.4.4 (January 2015), and EU25 + 3 (2000) for the normalization.

2.7. Statistical analysis

Data analysis and statistics (ANOVA) were performed using the Statistica 7.0 package using Tukey test with 95% confidence.

3. Results and discussion

3.1. Wastewater treatment plant effluent quality

The GreenDune PBRs were fed continuously with effluent after secondary treatment, named as "influent" from here onwards, to assess its efficacy for tertiary treatment. As this influent had already undergone secondary but not tertiary treatment, it presented low carbon and phosphorus contents and high concentrations of nitrogen, and there was a clear variation according to seasons for some parameters due to changes in temperature and rainfall (Table 2). Total phosphorus (TP) never exceeded 5 mg L^{-1} (lower than the 10 mg L^{-1} allowed by the discharge license of this WWTP, issued by the Portuguese Environmental Protection Agency) with the highest values observed in the winter but with no significant differences from the other seasons.

Total nitrogen (TN) varied between 20.2 mg L^{-1} in autumn and 61.3 mg L^{-1} in spring. All values exceed the discharge legal limits for TN (15 mg L^{-1}), highlighting the need for the tertiary treatment of these effluents. The main forms of nitrogen in wastewater after secondary treatment were nitrates (NO_3^--N) and ammonium (NH_4^+-N); nitrites (NO_2^--N) were assumed to be low as the oxygen concentration was

Table 2
Average composition of the influent in the different periods in which the experiments took place. The same letters indicate no statistical difference between conditions.

Nutrient (mg L^{-1})	Winter HRT 24 h	Winter HRT 48 h	Spring HRT 24 h	Spring HRT 12 h	Summer HRT 24 h	Summer HRT 48 h	Autumn HRT 24 h	Autumn HRT 48 h
TN	26.90 ± 2.50^{b}	21.98 ± 5.04^{b}	61.34 ± 15.28^{a}	35.21 ± 16.66^{a}	29.87 ± 12.37^{a}	20.16 ± 2.31^{a}	30.91 ± 14.77^{a}	20.25 ± 4.98^{a}
NO_3^-	31.90 ± 5.67^{c}	36.61 ± 9.22^{c}	2.74 ± 0.95^{d}	$53.01 \pm 31.59^{b,c}$	6.46 ± 5.32^{d}	10.68 ± 3.71^{d}	90.71 ± 12.17^{a}	65.5 ± 21.14^{b}
NH_4^+	21.65 ± 2.55^{b}	13.11 ± 3.86^{c}	30.63 ± 5.45^{a}	10.01 ± 5.50^{c}	29.08 ± 4.63^{a}	$18.01 \pm 2.59^{b,c}$	1.80 ± 1.37^{d}	0.51 ± 0.50^{d}
TP	4.98 ± 0.81^{a}	4.90 ± 0.75^{a}	1.83 ± 0.54^{a}	2.76 ± 0.36^{a}	2.07 ± 0.66^{a}	1.90 ± 0.41^{a}	2.00 ± 1.63^{a}	1.25 ± 0.32^{a}
PO_4^-	n.d.	n.d.	$5.55 \pm 3.36^{b,c}$	11.23 ± 1.85^{a}	$5.59 \pm 2.52^{b,c}$	5.14 ± 1.89^{c}	9.78 ± 1.17^{a}	$8.49 \pm 1.47^{a,b}$
N:P	12:1	10:1	74:1	28:1	32:1	23:1	34:1	88:1
COD	n.d.	n.d.	$63.14 \pm 25.80^{a,b}$	150.28 ± 57.30^{a}	111.70 ± 56.80^{a}	137.50 ± 31.04^{a}	28.90 ± 10.04^{b}	$90.25 \pm 54.12^{a,b}$

*n.d. not determined.

probably high as commonly seen in algae dominated cultures typical of microalgal tertiary treatments (Acién et al., 2016). The difference between TN and nitrates and ammonium was assumed to be organic nitrogen. NH_4^+-N varied from 0.512 mg NH_4^+ L^{-1} in autumn to 30.6 mg NH_4^+ L^{-1} in spring while NO_3^--N varied from 2.74 mg NO_3^- L^{-1} in spring to 90.7 mg NO_3^- L^{-1} in autumn. Higher concentrations of nitrates (and lower of ammonium) were especially evident in autumn and may be a result of an increase in sediments in the wastewater which, depending on the preceding dry weather flow, can build-up in the system during rainfall events increasing the abundance of ammonia-oxidizing bacteria and archaea (Wilén et al., 2006; Arce et al., 2018).

Each nutritional source can change the microbial consortia as well as the induction of specific conditions and chemical composition (Fallahi et al., 2021). For example, the N:P ratio can influence the synergistic interaction between microalgae and bacteria. In most of the experiments, microalgae were growing under phosphorus starvation and high nitrogen content, except in the winter experiment in which the N:P ratio was between 12:1 and 10:1. In the other seasons, the N:P ratios varied

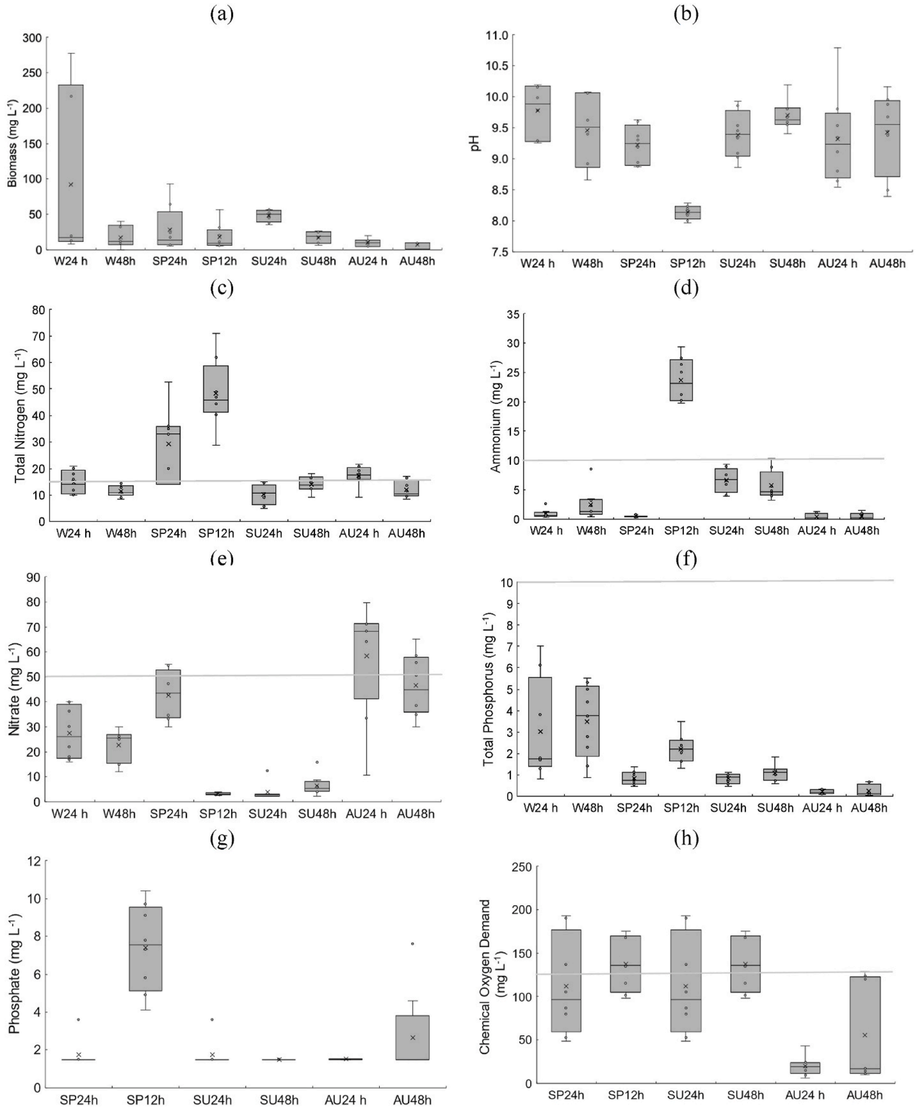

Fig. 2. Biomass concentration (mg L^{-1}) (a), pH variation (b), and water quality parameters in the PBRs effluent (treated wastewater) (c) TN, (d) NH_4^+, (e) NO_3^-, (f) TP, (g) PO_4^{3-} and (h) COD in the different seasons and hydraulic retention times: winter 24h (W24h), winter 48h (W48h), spring 24h (SP24h), spring 12h (SP12h), summer 24h (SU24h), summer 48h (SU48h), autumn 24h (AU24h) and autumn 48h (AU48h). In these diagrams, the x represents the average value and the horizontal dash the median of the nutrient's concentration. The box limits are defined by the lower and upper quartiles of the data and the whiskers are the minimum and maximum values of the data set. The horizontal lines across the charts represent the legal limit for that nutrient in the discharged treated wastewater.

from 23:1 to 88:1 (Table 2). The optimal ratio for microalgal growth according to Redfield (1960) is 16:1. Ratios of 5:1 to 12:1 are optimal for *Scenedesmus* sp. over other species as this species can display high growth rates even when the availability of nutrients, mainly P, is low (Arias et al., 2018).

COD values ranged from 29.9 mg O_2 L^{-1} to 150 mg O_2 L^{-1}, in some time points surpassing the legal limit of 125 mg L^{-1} (SP12h and SU48h).

3.2. Biomass concentration and treatment efficiency

The biomass concentration in the GreenDune PBRs was generally low and showed great variability between seasons (Fig. 2a). However, despite the bubble curtain of these PBRs that intends to limit biofilm formation in the photic zone, there is abundant formation of microalgal biofilm on the side and back walls of the PBRs making it extremely difficult to evaluate biomass production; it is, therefore, possible that the biomass concentrations shown in Fig. 2a are quite underestimated. Nevertheless, biofilms may play an important role in the removal of nutrients, as microorganisms within these films have access to the nutrients for prolonged periods of time, displaying better values in terms of nutrient uptake and sequestration (Biswas et al., 2021).

Biomass concentration in the PBRs was generally higher when an HRT of 24h was applied. This can be seen by analysing the median of the values for each season/HRT (the horizontal dash in each boxplot) which is higher for the summer (SU24h) experiment and by the higher dispersion of biomass concentration values observed in the winter (W24h) and spring (SP24h) experiments (Fig. 2a). In spring, when an HRT of 12h was used (SP12 h) the biomass concentration was very low as the residence time of the effluent in the reactors was probably too low, resulting in microalgal biomass washout. During the autumn experiment, a decrease in biomass production was observed due to the dilution of the cultures and nutrients in the wastewater caused by abundant rainfall (Table 1); this occurred for both HRTs tested (24h and 48h). In the winter, especially when an HRT of 24h was used, higher biomass concentration was observed (Fig. 2a). This might be due to the higher ammonia concentration (1.7 times higher) of the inflow together with an ideal N:P (12:1) ratio for microalgae growth (Table 2). In SP24h, despite a high ammonium concentration, TP was low resulting a very high N:P ratio (74:1; Table 2). According to Takabe et al. (2016), HRT has large effects on biomass yields; these authors recommend 48–72 h to obtain maximum biomass when working in temperatures between 12 and 25 °C with indigenous microalgae cultured in secondary effluent. In fact, in this study culture washout was observed when a 12h HRT was applied.

pH varied from 8 to 11 along the year (Fig. 2b). In autumn, even though there was a considerable reduction in biomass concentration (Fig. 2b), pH was always higher than 8, indicating that despite dilution caused by rainfall microalgae were actively growing. pH values are mainly related to the dissolution of the compounds on culture media, and increases in pH can be attributed to the consumption of inorganic carbon by microalgae during growth.

In general, pH values between 9 and 11 indicate a predominance of microalgae in the wastewater, mainly due to the fixation of CO_2 in organic molecules through photosynthesis. Decreased bacterial proliferation and an increase in microalgal proliferation can lead to an increase in pH (to values above 9) in the medium, due to a decrease in dissolved CO_2. Assimilation of nitrogen by microalgae also increases the pH value of the medium as every nitrate ion reduced to ammonia produces one OH^- (Chai et al., 2021).

Table 3 shows the removal rates of N and P nutrients while Fig. 2c–h shows nutrient and COD concentrations in the treated water. The removal rate of TN was always lower than 64%. This is probably a consequence of a poor sedimentation of the microalgal biomass in the settlers that were not properly sized. As TN is analysed in non-filtered samples, non-effective sedimentation could have led to an increased TN content in the treated water due to the presence of excess biomass (Fig. 2c). Ferro et al. (2019) using a microalgae-bacteria consortium to treat synthetic wastewater (70 mg L^{-1} TN) also observed a removal rate of total nitrogen of 69% for an HRT of 3d. In the spring (for both 24h and 12h HRT) and autumn 24h, most of the samples had TN values higher than the allowed limit for treated water discharge (15 mg N L^{-1}). This could be linked to the low biomass concentration in the PBRs observed in these seasons (Fig. 2a) either due to culture washout (SP12h) or because of culture dilution due to intense rain periods (autumn) or because of unusually high TN concentrations which rendered the N:P ratio unfavourably higher (SP24h) leading to P deficiency (Table 2).

The microalgal consortia of the GreenDunes generally displayed good removal rates for NH_4^+-N (65.7–95.4%), except in SP12h and in autumn 48h trial when the amount of NH_4^+-N was already very low in the influent (0.51 mg L^{-1} NH_4^+) (Table 3). In fact, except for the SP12h experiment, in all the other trials the treated water had NH_4^+-N values below the legal limit of 10 mg L^{-1} (91/271/EEC; Fig. 2d).

Nitrates (NO_3^--N) removal rates were lower than those of NH_4^+-N; however, in most experiments (Table 3), the system was able to meet the legal requirements of 50 mg L^{-1} (91/271/EEC; Fig. 2e). The only exception were the autumn experiments in which the influent contained the highest values of NO_3^--N with high N:P ratios (Table 2). Even when high removal rates were observed, NO_3^--N values in autumn season and at an HRT of 24h were still above legal discharge limits; however, that was not the case when an HRT of 48h was used. In this case, both the average and median NO_3^--N values were below legal limits.

Removal of nitrogen and phosphorus compounds (Table 3) was highest for NH_4^+, PO_4^{3-} and TP, independently of the season and HRT, with removal values higher than 70% (Table 3). The removal rates of phosphorus could be directly related to the N:P ratio, the higher the ratio the higher TP removal values. In all the experiments, N:P ratio was always higher than 10:1 (Table 2) and hence cultures were never nitrogen deficient leading to good P removals (Table 3). The concentration of phosphates in the treated water is not limited by law but similarly to TP, high removal rates (between 68.7 and 84.3%) were observed for PO_4^{3-}-P (Table 3) and the concentration in the treated water was generally low (<4 mg L^{-1}), except for the SP12h experiment, similarly to what occurred for the remaining nutrients (Fig. 2).

COD was low even in the influent, characteristic of an effluent after secondary treatment. However, in some limited cases, the effluent of the GreenDune system surpassed the legal limit of 125 mg O_2 L^{-1} (Fig. 2h). The settlers used in this study to separate microalgal biomass from treated water were not efficient leading to poor sedimentation of the microalgal biomass and an excess amount of suspended cells were still present in the treated water. As COD analyses are performed in non-filtered samples microalgae present in treated water may contribute to

Table 3
Nutrients removal (%) from the effluents in different seasons and hydraulic retention times. The same letters indicate no statistical difference between conditions.

	Winter 24 h	Winter 48h	Spring 24h	Spring 12h	Summer 24h	Summer 48h	Autumn 24h	Autumn 48h
TN	$44.3 \pm 17.2^{a,b}$	$43.7 \pm 15.9^{a,b}$	18.8 ± 22.0^{b}	23.1 ± 15.6^{b}	64.2 ± 15.4^{a}	33.1 ± 14.6^{b}	$42.3 \pm 19.3^{a,b}$	$42.6 \pm 12.8^{a,b}$
NO_3^-	32.7 ± 19.9^{a}	45.3 ± 19.1^{a}	33.2 ± 8.7^{a}	n.r.	39.9 ± 18.9^{a}	50.2 ± 25.2^{a}	42.8 ± 29.3^{a}	34.0 ± 17.3^{a}
NH_4^+	95.4 ± 4.1^{a}	$82.4 \pm 15.7^{a,b}$	65.7 ± 25.5^{a}	24.9 ± 8.6^{c}	$76.0 \pm 9.3^{a,b}$	$72.4 \pm 11.1^{a,b}$	$75.0 \pm 23.9^{a,b}$	n.r.
TP	$33.4 \pm 27.5^{a,b,c}$	51.5 ± 32.4^{b}	$70.4 \pm 11.6^{a,b}$	n.r.	$61.6 \pm 19.4^{a,b,c}$	$43.7 \pm 21.7^{b,c}$	84.8 ± 13.8^{a}	86.0 ± 17.6^{a}
PO_4^{3-}	n.d.	n.d.	83.7 ± 7.9^{a}	n.r.	68.7 ± 13.8^{b}	69.4 ± 9.4^{b}	84.3 ± 1.8^{a}	$77.2 \pm 9.6^{a,b}$

*n.r. not removed; *n.d. not determined.

an elevated COD value. Nonetheless, all median and average levels were below legal limits.

3.3. *Wastewater reuse*

The analysis of the scenarios for possible reuse of treated wastewater was made considering the medians and range of the values obtained (Table 4) and no significant differences were observed between the studied scenarios (Tables 5 and 6). The application of a conservative scenario (CS) makes the reuse of wastewater unfeasible, both for an HRT of 24 or 48h (see Tables S1, S2 and S3 in Supplementary information). However, application of a non-conservative scenario (NCS) allows the reuse of treated wastewater for seed production, irrigation of naturally restricted use areas (e.g., hedges, containment areas, terraced meadows) if PBRs are operated at an HRT of 48h. The parameter with the highest impact on reuse was *E. coli.* which values limits the reuse of water in most of the reuse scenarios considered. *E. coli* concentration was considerably higher when an HRT of 48h was applied. However, the conditions for which microalgal systems are considered to contribute to wastewater disinfection (e.g., high light penetration that promotes UV disinfection and high pH that inhibit bacterial growth) were similar in both the 24 and 48h experiments. The only difference that could possibly account for higher bacterial growth is the BOD_5 which was higher in the 24h experiment. Nonetheless, there are only two situations (reclaimed water quality class/uses) in which the operation of the system with an HRT of 24h performed better than at 48h, concerning the parameter *E. coli*: Class C of agricultural use and for industrial use in circuits with direct risk of dermal contact (both under a CS scenario). TSS negatively impacted the reuse of the treated wastewater for agricultural and irrigation of urban spaces without and with access restriction, while pH affected the urban use cases; in turn, turbidity limited water reuse in both urban and industrial use cases (Tables 5 and 6). In addition, if the CS is considered, urban recreational reuse was limited also for the HRT of 48h, due to high NH_4^+-N and TP concentrations.

Microbiological parameters are a major issue regarding wastewater reuse (WHO, 2006) and microalgae treatment has the potential to reduce bacterial concentration through increased sunlight exposure, higher dissolved oxygen concentration and high pH, for example (García et al., 2008). Kotoula et al. (2020) used a combined system of microalgae *Chlorella sorokiniana* and the macrophyte *Lemna minor* for municipal wastewater treatment, in sequenced batch experiments. Results demonstrated that wastewater could be reused for irrigation considering the COD, NH_4^+-N, TN and TP. However, TSS and pathogens were not evaluated, and the disinfection of the effluent before reuse was recommended (Kotoula et al., 2020). A similar analysis was made by Morillas-; España et al. (2021) using *Scenedesmus* sp. in two pilot-scale thin-layer cascade photobioreactors. Overall results indicated that treated wastewater could be reused for agricultural irrigation or disposed into water courses, but no evaluation was made for microbiological parameters, BOD_5, TSS and pH (Morillas-España et al., 2021).

In the case of the present study, some operational optimisation is still needed, especially to decrease TSS, turbidity and pH values. The sedimentation process for liquid-solid separation of the microalgae must be improved, which will decrease the TSS and turbidity. pH between 6 and 9 is necessary for urban reuses (Table S2). This may require management options. For example, pH values above 9 may guarantee high removals of pathogens (Rani et al., 2021) such as *E. coli*, but the pH of treated water must be decreased via, for example, addition of acidifying chemicals. Alternatively, the maintenance of pH in the required range, for example, by applying CO_2 to the microalgal cultures, may increase treatment efficiency for nutrient removal but the disinfection of the treated wastewater will then be needed (Posadas et al., 2015).

Table 4
Median and range of parameters measured in the treated water during the winter campaign for both HRT tested.

Parameters	HRT 24 h		HRT 48 h	
	Median	Range	Median	Range
pH	9.54	8.87–10.20	10.01	8.66–10.29
BOD_5 (mg L^{-1})	13.33	7.33–17.33	8.67	7.33–11.33
TSS (mg L^{-1})	76.17	41.33–141.17	40.93	27.60–83.67
Turbidity (NTU)	29.02	14.85–55.46	14.69	2.27–32.07
E. coli (cfu 100 mL^{-1})	671	5–1337	4073	7–5187
NH_4^+ (mg L^{-1})	0.7	0.3–2.6	1.3	0.4–8.6
TN (mg L^{-1})	14.0	10.0–20.8	11.1	8.4–15.4
TP (mg L^{-1})	1.8	0.8–7.0	3.8	0.9–5.5
Salinity (dS m^{-1})	–	0.9	–	0.9

The United Nations sustainable development goal 6 (SDG6) is to substantially increase water-use efficiency across all sectors by 2030 (UN, https://sdgs.un.org/goals/goal6). In addition, the full value of water can be recognized and captured in circular economy (Sharma et al., 2021). Thus, the approach here presented is an important contribution towards the worldwide goal of promoting treated water reuse by using microalgae and a novel photobioreactor design.

3.4. *Biomass chemical characteristics and possible application*

Taking into consideration the average quantity of raw wastewater at Quinta do Lago WWTP is 1.789.485 m^3 $year^{-1}$, and an average biomass concentration of 50 mg L^{-1}, this WWTP has the potential to produce around 89 ton of biomass per year. The valorization of this biomass could therefore help to mitigate the costs associated with wastewater treatment. The composition of the biomass varied according to season rather than due to changes in HRT, especially in what concerns lipids that were higher in the summer regardless of the HRT applied (Fig. 3). This is probably due to the variations in light, temperature, and wastewater chemical composition that influenced the biodiversity dynamics of the microalgae-bacteria consortia, thus changing the biomass biochemical profile.

The highest lipid accumulation was observed in the summer regardless of the applied HRT (Fig. 3). This may be due to the high temperatures and light exposure observed during this season (Table 1), which could have been a stressful condition for microalgal growth, shifting the metabolism of microalgae from carbohydrates and protein production to lipids production (Markou and Nerantzis, 2013). Lipids were always the least abundant macronutrient in the collected biomass which is probably related with the fact that wastewater is rich in nitrogen, therefore leading to a biomass richer in protein. Jiang et al. (2016) also observed an increase in the protein levels when the microalga *Monoraphidium* spp. SDEC-1 was cultivated in ammonia-rich wastewater (from 35% in BG-11 to 44% in wastewater). Conversely, nitrogen starvation is usually applied to microalgal cultures to increase the lipid levels at the expense of protein content (Gojkovic et al., 2020). Lipid-rich biomass (>20% of dry biomass) (Chisti, 2008) could be used for biodiesel production, especially biomass rich in saturated non-polar lipids (Gangadhar et al., 2016). However, given the low lipid content of the biomass obtained in these experiments (from 5.43 to 12.8%), its use for biodiesel production is not recommended.

In the produced biomass, ash content varied between 20.0% (SU24h) and 44.7% (SP12h). Other authors also observed high ashes content in microalgae grown in different effluents like piggery (39.0%, Silveira et al., 2021) and domestic (20.5%, Assis et al., 2020) wastewaters. According to these authors, wastewaters have suspended particles and metals that can contribute to this high ash content.

Protein content in the cultures varied from 36.3% (W24h) to 23.2% (SP12h), however few significant differences were observed as apart from the lower value obtained in SP12 protein values were relatively constant (Fig. 3).

Carbohydrates were the component that varied the most with HRT, only in the summer when it ranged from 35.6% (SU48h) to 19.3% (SU24h). This large variation could be due to the high temperatures registered in this season. Stressful culture conditions such as high

Table 5
Treated wastewater reuse possibilities according with Portuguese Law decree n° 119/2019 (HRT 24 h).

Reclaimed water quality class/Quality level	pH		BOD_5 (mg L^{-1})		TSS (mg L^{-1})		Turbidity (NTU)		*E. coli* (cfu 100 mL^{-1})		Parasites intestinal eggs (no L^{-1})		NH_4^+-N (mg L^{-1})		TN (mg L^{-1})		TP (mg L^{-1})	
	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS
Agricultural use																		
A/High	n.a.	n.a.	X	X	X	X	X	X	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
B/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
C/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	✓	X	n.d.	n.d.	✓	✓	✓	x	✓	x
D/Low	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	✓	✓	n.d.	n.d.	✓	✓	✓	x	✓	x
E/Low	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	✓	✓	x	x	✓	✓	✓	x	✓	x
Urban use																		
Irrigation without access restriction/High	n.a.	n.a.	X	X	X	X	X	X	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
Recreational uses/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	✓	✓	n.a.	n.a.	✓	X
Firefighting water/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	X	X	n.a.	n.a.	n.a.	n.a.
Toilet water/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	✓	✓	n.a.	n.a.	n.a.	n.a.
Street washing with manual high-pressure systems/High	X	X	✓	✓	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Vehicle wash with manual high-pressure systems/High	X	X	n.a.	n.a.	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Irrigation with access restriction/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
Cooling water/Medium	X	X	✓	✓	n.a.	n.a.	n.a.	n.a.	X	X	n.a.	n.a.	✓	✓	n.a.	n.a.	n.a.	n.a.
Industrial use																		
In circuits with direct risk of ingestion and dermal contact/Medium	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
In circuits with direct risk of dermal contact/Medium	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	X	X	X	✓	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

NCS – Non-conservative scenario; CS – Conservative scenario; (n.d.) not determined; (n.a.) not applicable because it is not part of legislation; (✓) According with referred values; (X) Not according with referred values, smaller symbols indicate that the parameter is facultative since it is not a mandatory parameter, it is just a recommendation.

temperature and high light are known to trigger metabolic changes, including degradation of intracellular protein and pigments and accumulation of storage compounds such as carbohydrates and lipids (Jung et al., 2019). In fact, samples collected in the summer, when higher temperatures were observed (Table 1), contained significantly higher amounts of lipids (Fig. 3). Additionally, the amount of carbohydrates in the biomass can be highly influenced by the N:P ratio of the cultures (Solís-Salinas et al., 2021). Sánchez-Contreras et al. (2021), using a mixed microalgae culture to treat industrial wastewater, achieved the highest carbohydrate content (57%) in the culture with P limitation and a N:P ratio of 22:1 HRT of 8d, a similar ratio to the one the reach the higher carbohydrate content in this study (23:1, SU48h). Possible applications for the biomass produced in these conditions could be the production of biofuels as biogas (Jones and Mayfield, 2012), or biohydrogen (Sharma et al., 2021). Especially biogas, given the percentage of proteins and carbohydrates in the biomass obtained for all different HRT and seasons, could be a feasible application.

3.5. *Environmental impacts of the new technology*

The environmental impacts of the GreenDune system were determined assuming the need to size a facility to replace the nitrification-denitrification operation at the Quinta do Lago WWTP. The impacts of the new technology are benchmarked against the unit currently installed at the WWTP. Sizing and inventory data are provided in Table S4 (Supplementary Material).

The construction methods used in the two alternatives are substantially different (Table S4 in SM). Most important material flows include reinforced concrete (ca. 62 tones) and steel piping (100 kg) for nitrification/denitrification, whereas for the GreenDune PBRs they are polyacrylate sheets (ca 450 tones) and PVC piping (1040 kg). Despite the contrasting construction methods, the impacts measured by the different categories during the construction phase showed to be similar in the two treatment alternatives (Fig. S2 in Supplementary Material). E.g., for the two categories with the highest magnitudes, they were: Global warming potential (6.56 and 6.61 log kg CO_2 eq, respectively) and Marine aquatic ecotoxicity (8.10 and 7.97 log kg 1,4-DB eq, respectively).

During operation, the most important flows are methanol (ca 21 400 kg/month) and electricity (ca 123 000 kWh/month) for nitrification/denitrification. Electricity is the only flow in the new solution (ca 42 000 kWh/month). Again, for the two categories with the highest magnitudes, they were: Global warming potential (7.76 and 7.29 log kg CO_2 eq, respectively) and Marine aquatic ecotoxicity (9.38 and 8.31 log kg 1,4-DB eq, respectively). The consumption of methanol and the pumping electricity consumption justify the difference in favor of the new solution.

Globally, the GreenDune system showed total impact magnitudes for the sum of construction and operation phases between two and three times lower than those of the nitrification-denitrification unit (Table 7).

4. Conclusions

The new Greendune PBRs were able to perform the tertiary treatment of urban wastewater in different seasons using an HRT of up to

Table 6
Treated wastewater reuse possibilities according with Portuguese Law decree n° 119/2019 (HRT 48 h).

Reclaimed water quality class/Quality level	pH		BOD_5 (mg L^{-1})		TSS (mg L^{-1})		Turbidity (NTU)		*E. coli* (cfu 100 m L^{-1})		Parasites intestinal eggs (n° L^{-1})		NH_4^+-N (mg L^{-1})		TN (mg L^{-1})		TP (mg L^{-1})	
	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS	NCS	CS
Agricultural use																		
A/High	n.a.	n.a.	✓	n.a	X	X	X	X	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
B/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
C/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	X	X	n.d.	n.d.	✓	✓	✓	x	✓	x
D/Low	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	✓	✓	n.d.	n.d.	✓	✓	✓	x	✓	x
E/Low	n.a.	n.a.	✓	✓	✓	X	n.a.	n.a.	✓	✓	n.a.	n.a.	✓	✓	✓	x	✓	x
Urban use																		
Irrigation without access restriction/High	n.a.	n.a.	✓	X	X	X	X	X	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
Recreational uses/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	✓	X	n.a.	n.a.	X	X
Firefighting water/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Toilet water/High	X	X	✓	✓	n.a.	n.a.	X	X	X	X	n.a.	n.a.	✓	✓	n.a.	n.a.	n.a.	n.a.
Street washing with manual high-pressure systems/High	X	X	✓	✓	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Vehicle wash with manual high-pressure systems/High	X	X	n.a.	n.a.	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Irrigation with access restriction/Medium	n.a.	n.a.	✓	✓	X	X	n.a.	n.a.	X	X	n.a.	n.a.	✓	✓	✓	x	✓	x
Cooling water/Medium	X	X	✓	✓	n.a.	n.a.	n.a.	n.a.	X	X	n.a.	n.a.	✓	X	n.a.	n.a.	n.a.	n.a.
Industrial use																		
In circuits with direct risk of ingestion and dermal contact/Medium	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
In circuits with direct risk of dermal contact/Medium	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	X	X	X	X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

NCS – Non-conservative scenario; CS – Conservative scenario; (n.d.) not determined; (n.a.) not applicable because it is not part of legislation; (✓) According with referred values; (X) Not according with referred values, Smaller symbols indicate that the parameter is facultative since it is not a mandatory parameter, it is just a recommendation.

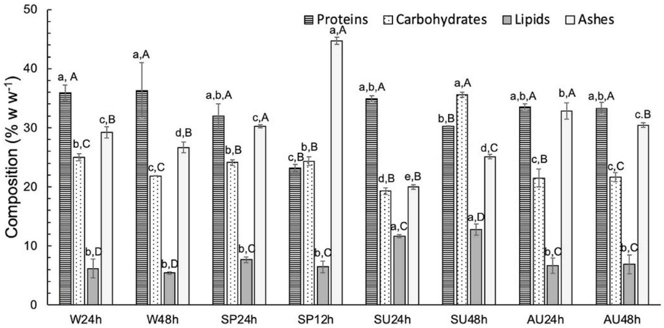

Fig. 3. Chemical composition of the biomass produced (dry basis) in the different seasons and HRT tested: winter 24h (W24h), winter 48h (W48h), spring 24h (SP24h), spring 12h (SP12h), summer 24h (SU24h), summer 48h (SU48h), autumn 24h (AU24h) and autumn 48h (AU48h). The same lower-case letters indicate no significant differences between seasons. The same capital letters indicate no significant differences between HRT.

Table 7
Comparison of lifetime impacts for GreenDune and nitrification-denitrification. Values in Log10 units.

Impact category	Nitrification-denitrification (ND)	GreenDune (GD)	ND/GD (%)
Abiotic depletion (kg Sb eq)	1.90	1.46	276
Acidification (kg SO_2 eq)	5.61	5.18	272
Eutrophication (kg PO_4^- eq)	4.49	4.07	263
Freshwater aquatic ecotoxicology (kg 1,4-DB eq)	5.72	5.26	291
Global warming (GWP100) (kg CO_2 eq)	7.78	7.37	258
Human toxicity (kg 1,4-DB eq)	7.02	6.62	249
Marine aquatic ecotoxicity (kg 1,4-DB eq)	9.40	8.96	277
Ozone layer depletion (ODP) (kg CFC-11 eq)	~0	~0	–
Photochemical oxidation (kg C_2H_4 eq)	3.24	2.96	190
Terrestrial ecotoxicity (kg 1,4-DB eq)	4.96	4.51	278

48h. The system was able to remove up to 95% of NH_4^+, the main pollutant in wastewater after secondary treatment using 24h of HRT (winter season). Removal of nitrogen and phosphorus-containing compounds was highest for NH_4^+, PO_4^{3-} and TP, independently of the season and HRT. Treated water could be reused for seed production or irrigation of naturally restricted use areas, but other possible uses can be envisaged if a more efficient settling system is applied. Compared with the nitrification/denitrification currently existing at the WWTP, the GreenDune system presents higher benefits for the environment as its operation represents an environmental impact two or three orders of magnitude lower than the currently used system. However, the GreenDune PBRs will not be able to completely substitute conventional WWTP as the retention times and hence the occupied land area is still high. However, the analysed algal system can be used in combination with conventional treatment, reducing environmental impacts and treatment costs as a biomass rich in valuable with potential to produce biofertilizers and biofuels is obtained. Another possibility could be to implement this algal system in remote rural areas or in developing countries, which in most cases do not have an adequate treatment system and where land is usually available.

CRediT authorship contribution statement

Etiele Greque de Morais: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. **José Carlos Amaro Marques:** Investigation. **Paulo Ricardo Cerqueira:** Investigation. **Cláudia Dimas:** Investigation. **Vânia de Sousa:** Investigation. **Nuno Gomes:** Resources, Conceptualization, Methodology, Funding acquisition. **Margarida Ribau Teixeira:** Writing – original draft, Resources, Validation, Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision. **Luís Miguel Nunes:** Writing – original draft, Resources, Investigation, Conceptualization, Methodology, Writing – review & editing, Visualization, Funding acquisition. **João Varela:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Luísa Barreira:** Validation, Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.134521.

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