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Assessment of the influence of the hydraulic retention time on the tertiary and quaternary treatment of a high rate algal pond

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ABSTRACT

The influence of the hydraulic retention time (HRT) on the performance of a high-rate algal pond (HRAP) with biomass settling and recycling for tertiary and quaternary treatment of domestic wastewater was herein evaluated. A decrease in the HRT from 4 days (P1) to 2 days (P2) did not entail substantial differences in the removal efficiencies of nitrogen (54.3 ± 11.6 and 54.0 ± 7.3 %), phosphorous (42.6 ± 21.6 and 39.3 ± 35.8 %), organic carbon (33.5 ± 12.2 and 38.4 ± 26.4 %) and inorganic carbon (55.6 ± 10.8 and 50.5 ± 26.8 %). *Escherichia coli* (0.7 ± 1.4 and 3.8 ± 5.2 CFU-mL^{-1}, respectively) and suspended solid (10 mg TSS-L^{-}1 average) concentrations in the effluent remained below European discharge limits. However, the effluent total nitrogen (TN) concentration exceeded those limits, despite the high NH_4^+ removal capacity ($>$ 95 %) of the HRAP. Regarding CECs, 9 compounds were removed below their quantification limit in P1, and 5 compounds in P2. The HRT exhibited a low impact on the total removal of CECs, with removal efficiencies of 57.6 ± 4.0 % and 63.7 ± 3.1 % during P1 and P2, respectively. The CEC concentrations retained in biomass increased at decreasing the HRT, with 723 and 1026 μg L^{-1} at P1 and P2, respectively. Most of these compounds belonged to the group of fluoroquinolones or anti-inflammatory drugs. Overall, HRAPs represent a cost-effective technology platform for tertiary and quaternary treatment of domestic wastewaters.

1. Introduction

Domestic wastewater treatment in most developed countries is nowadays carried out using conventional activated sludge processes based on mechanical aeration via air bubbling or superficial turbines. The high energy consumption of this aeration typically results in high operating costs (45–75 % of the total cost) and a high environmental impact due to indirect CO_2 emissions [1,2]. Moreover, the limited nutrient assimilation in the form of biomass in activated sludge processes supports a low nutrient recovery from wastewater [3], which leads to a loss of these valuable resources present in domestic wastewater.

On the other hand, there is a growing concern about the environmental and health related impacts of the so-called contaminants of emerging concern (CECs), which are persistent synthetic organic chemicals capable of damaging the environment at very low concentrations (nano to milligrams per liter). Unfortunately, conventional activated sludge processes provide an insufficient elimination of these CECs (e.g., pharmaceutical compounds, analgesics, pesticides, etc.), which nowadays limits the reuse of treated water [4–6]. This limited treatment performance of conventional systems also extends to other relevant processes such as the removal of pathogens from treated water (i.e. fecal bacteria or other microorganisms that may be hazardous to human health) [7]. Therefore, conventional secondary treatment typically results in an effluent characterized by a low organic matter content and high concentration of nitrates and nitrites along with above-limit concentrations of phosphates [8,9] and other relevant contaminants like pathogens [10] and CECs [11], which must be removed before being disposed into the environment.

Regarding the above-mentioned problematic, microalgae-based processes represent a sustainable proposal for supporting conventional activated sludge processes as a next step for the treatment of domestic

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wastewater [12]. Wastewater treatment in algal-bacterial photobioreactors has been based on the intensification of the symbiosis between photosynthetic microalgae and heterotrophic/nitrifying bacteria. In these systems, the photosynthetic oxygen produced by microalgae is used by aerobic heterotrophic microorganisms (bacteria or heterotrophic microalgae) to oxidize organic matter, producing the CO_2 that microalgae need to carry out photosynthesis in the presence of sunlight. In this context, nitrifying bacteria also use inorganic carbon as a carbon source and oxygen as electron acceptor for NH_4^+ oxidation. The assimilation in the form of algal-bacterial biomass of the CO_2 produced during the oxidation of organic pollutants and the inorganic carbon inherently present in the wastewater leads to a higher recovery of nutrients (N, P), and a decrease in the carbon footprint of the wastewater treatment process [13–15]. The latter represents the basis of the tertiary treatment of domestic wastewater. In this context, an effective mixing of the algal cultivation broth can promote photosynthesis and support the high pH and dissolved O_2 concentrations needed to boost pathogen removal from treated wastewater [7,16]. Unfortunately, there is a limited knowledge about the potential of algal-bacterial photobioreactors to remove pathogens from treated domestic wastewater.

Furthermore, algal-bacterial photobioreactors exhibit a series of unique biological and abiotic mechanisms that could eventually support a superior CEC elimination compared to activated sludge processes: i) an effective photolysis mediated by solar UV penetration in the shallow open ponds, ii) bioadsorption of CECs on the extracellular walls of microalgae or in compounds (such as polysaccharides) released by microalgae, iii) bioaccumulation (or bio-uptake) supporting the storage of CECs inside the cells and their subsequent removal with the wasted biomass; iv) biodegradation of CECs by algal-bacterial biomass mediated by the action of enzymes and v) hydrolysis mediated by the high pH and dissolved oxygen concentrations prevailing in the algal cultivation broth. Despite the potential of algal-bacterial photobioreactors for CECs removal, the number of studies assessing CECs fate during microalgae-based quaternary treatment is still scarce, which will help adopting this nature-based technology as an integrated part of a multi-stage wastewater treatment system. There is also a lack of information on how the removal of multi-pollutants evolves, and on their interaction and competition during tertiary/quaternary microalgae-based removal processes, since most studies using algal-bacterial systems were devoted to secondary/tertiary treatment [11,17]. Furthermore, there is a lack of information regarding the capacity of these systems to operate effectively under hydraulic retention times (HRTs) that are practical for tertiary treatment applications (i.e., 1–2 days), while secondary HRAP-based treatment systems typically require 4–10 days of HRT [9].

This work evaluated the performance of a microalgae-based pond implemented as a tertiary and quaternary treatment of real domestic wastewater previously treated in a conventional denitrification-nitrification activated sludge plant. In this regard, the influence of hydraulic retention time (HRT) on different process parameters was evaluated, including: (1) carbon and nutrient removal; (2) fecal pathogen removal; and (3) CECs removal (both by biodegradation and bioadsorption/bioaccumulation on the biomass).

2. Methodology

2.1. Inoculum and feedstock

A consortium of microalgae was used as inoculum for the start-up of the HRAP. This consortium was obtained from a high-rate algal pond (HRAP) operated outdoors at the Institute of Sustainable Processes of University of Valladolid (Valladolid, Spain). The main photosynthetic species of the inoculum were *Pseudoanabaena* sp. (98 %), a cyanobacterium genus, and *Chlorella vulgaris* (2 %), a green microalga specie. The feedstock used as substrate was the effluent of a nearby wastewater treatment plant (WWTP) (Valladolid, Spain), which is operated based on a denitrification-nitrification activated sludge process. This treated wastewater was weekly collected in 20 L batches and stored at 4 °C prior use. The composition of this treated wastewater is shown in Table 1.

2.2. Contaminants of emerging concern and reagents

The 44 target CECs are listed in Table 3. These compounds are grouped into different families: 22 antibiotics, 6 anti-inflammatories, 3 lipid regulators, 2 beta-blockers, 1 psychiatric drug, 1 antiparasitic, 1 hormone, 1 insect repellent, 1 pesticide, 4 cosmetic preservatives, 1 stimulant and 1 antipruritic. The selection of these compounds was based on their high consumption nowadays, and their ability to maintain their pharmacological potential in the environment. These compounds have been reported to be, to a greater or lesser extent, persistent or poorly degraded in conventional WWTP [18,19]. For a more accurate quantification on the analytical instrumentation, i.e., tandem mass spectroscopy (MS/MS), the following isotopically-labelled internal standards were used: methylparaben-d4, ethylparaben-d5, propylparaben-d7, naproxen-d3, diclofenac-d4, ibuprofen-d3, salicylic acid-d_4, ciprofloxacin-d8, sulfadimidine-d4, sulfamethoxazole-d4, sulfadiazine-d4, florfenicol-d3, progesterone-d9, carbamazepine-d10, caffeine-d10, trimethroprim-d3 and clofibric acid-d_4.

2.3. Experimental set-up and operating conditions

A pond mimicking a HRAP system (Fig. 1) was set up (in an indoor laboratory) with a total volume of 11.25 L and a working volume of 9 L. The reactor consisted of a polypropylene prism with a rectangular base (30 cm × 15 cm) and a height of 25 cm (average water layer of HRAPs). Two submerged pumps (model Eheim Compac 300) were used to maintain the algal-bacterial broth in suspension. In order to mimic a real process, the HRAP was in direct contact with the atmosphere and exposed to a photosynthetic active radiation (PAR) similar to peak solar radiation (1307 $\mu mol\ m^{-2}\ s^{-1}$) by using visible and UV LEDs (360–365 nm and 380–385 nm; 40 $w\ m^{-2}$) operated under light:dark cycles of 12 h:12 h. A Watson Marlow 120S pump was used to supply the wastewater to the HRAP. The reactor effluent was collected in a settler of 1 L capacity (Fig. 1c). A fraction of the settled biomass was recirculated back to the reactor to maintain a high biomass concentration (0.9 ± 0.2 and 1.0 ± 0.3 g/L at P1 and P2, respectively), while another fraction of the settled biomass was harvested periodically to maintain the mean cell residence time (MCRT).

Table 1
Average pollutant concentrations in the influent and effluent of the HRAP system. Removal efficiencies are calculated considering the evaporation of the system.

	Influent (mg L^{-1})	Effluent (mg L^{-1})	Removal (%)
PERIOD 1[a]			
TOC	17.1 ± 3.1	13. ± 1.9	33.5 ± 12.2
IC	86.2 ± 13.1	46.0 ± 8.4	55.6 ± 10.8
TN	36.9 ± 4.7	20.2 ± 4.5	54.3 ± 11.6
NH_4^+	27.5 ± 8.0	1.31 ± 1.1	95.7 ± 4.4
NO_2^-	14.5 ± 19.5	8.69 ± 4.1	49.99
NO_3^-	48.6 ± 30.6	66.9 ± 20.0	−15.5
PO_4^{3-}	2.55 ± 2.9	1.19 ± 0.6	42.6 ± 21.6
PERIOD 2			
TOC	16.9 ± 4.3	12.7 ± 4.4	38.4 ± 26.4
IC	57.3 ± 27.4	31.9 ± 13.8	50.5 ± 26.8
TN	38.1 ± 11.2	22.4 ± 5.4	54.0 ± 7.3
NH_4^+	14.6 ± 17.4	0.98 ± 1.5	97.5 ± 3.2
NO_2^-	27.1 ± 25.1	17.7 ± 8.4	41.61
NO_3^-	74.5 ± 37.9	64.8 ± 19.5	22.08
PO_4^{3-}	1.22 ± 0.63	0.59 ± 0.29	39.3 ± 35.8

[a] Period 1 and Period 2 correspond to HRT values of 4 and 2 days, respectively.

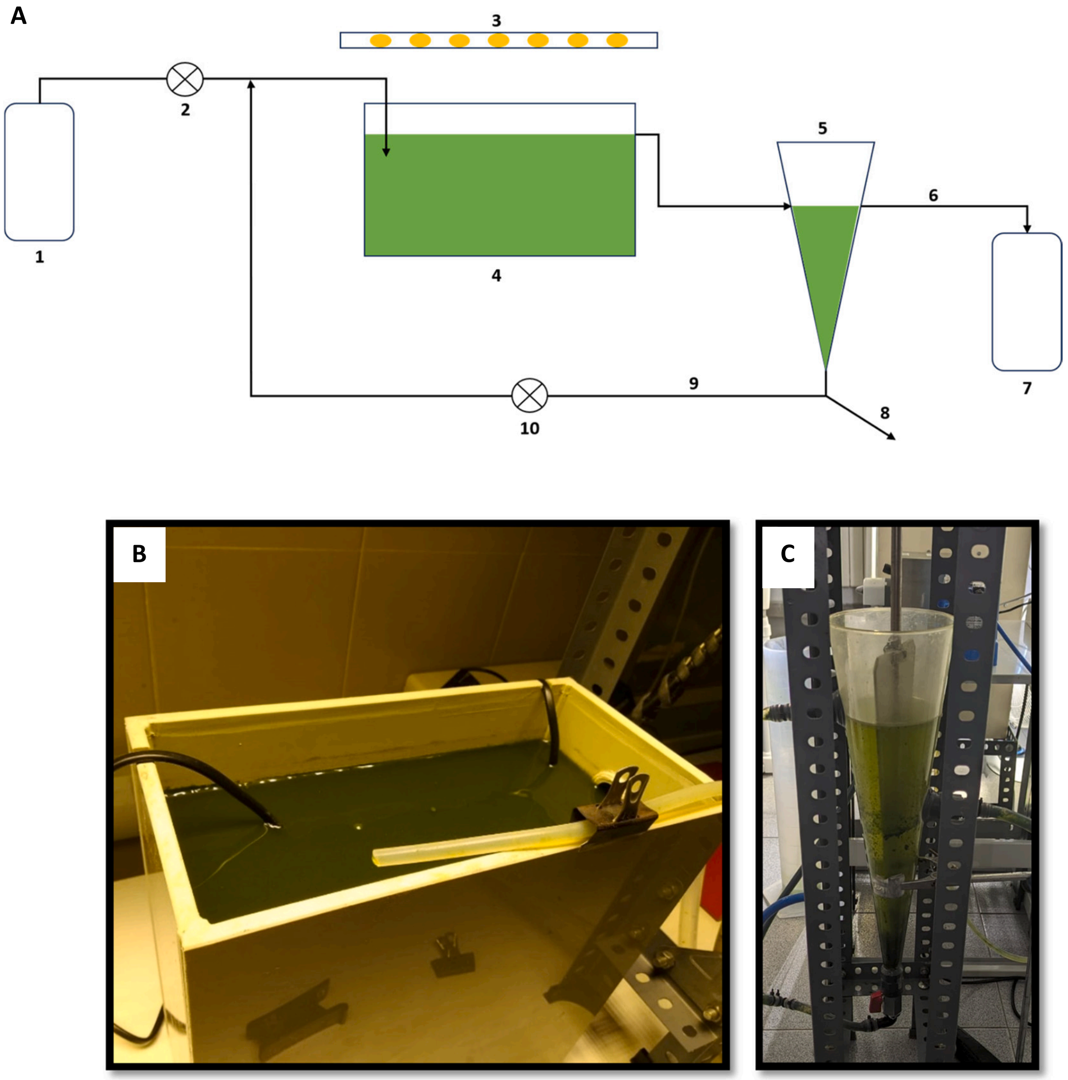

Fig. 1. A) Schematic diagram of the experimental HRAP system; B) Image of the HRAP; C) Image of the settler. Legend: Feedstock tank (1), Pumps (2 and 10), LED lights system (3), HRAP (4), Settler (5), Effluent stream (6), Effluent tank (7), Purge stream (8) and Recirculation stream (9).

2.4. Evaluation of the influence of the HRT on the HRAP performance

The HRAP was operated for 108 days to evaluate the effect of HRT on the removal efficiency of carbon, nutrients, CECs and pathogens. The HRAP was operated during Period 1 (P1) for 77 days (corresponding to 22 days of start-up period (data not shown) and 55 days of process) at 4 days of HRT (corresponding to an influent flow rate of 1.6 mL min^{-1}) and during period 2 (P2) for 31 days at 2 days of HRT (corresponding to an influent flow rate of 3.2 mL min $^{-1}$). The external recirculation from the bottom of the settler to the HRAP was paced at 50 % of the influent flowrate at both conditions. The steady states in P1 and P2 were maintained for at least 3 weeks. The influent and effluent concentrations of total organic carbon (TOC) and inorganic carbon (IC) were measured twice a week, along with the concentrations of total nitrogen (TN), NH$_4^+$, NO$_2^-$, NO$_3^-$, PO$_4^-$, Cl$^-$ and SO$_4^{2-}$. Total and volatile suspended solids (TSS and VSS, respectively) concentrations and pH were quantified in the influent, effluent, HRAP cultivation broth and waste biomass stream. The dissolved O$_2$ concentration (DO) and the temperature in the cultivation broth of the HRAP were measured twice a week along with chemical parameters. The influent and effluent concentration of *Escherichia coli* was measured once a week. Finally, the concentrations of CECs in the influent, effluent and settled biomass were determined under steady state in P1 and P2.

2.5. Analytical procedures

Temperature and DO were measured using an OXI 3310 oximeter (WTW, Germany). The pH was determined by using an Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands). PAR radiation was quantified using an LI-250a light meter (LI-COR Biosciences, Germany). A Shimadzu TOC-VCSH analyser (Japan) coupled with a TNM-1 chemiluminescence module was used for TN, TOC and IC determinations, from non-filtered samples (dissolved plus particulate forms). TSS and VSS concentrations were determined according to standard methods [20]. The concentrations of dissolved S-SO$_4^{2}$, N-NO$_2^-$, N-NO$_3^-$, PO$_4^{3-}$ and Cl$^-$ were determined by HPLC-IC (Waters 432, conductivity detector, USA) according to Posadas et al. (2013) [21]. Dissolved N-NH$_4^+$ concentration was determined by the Nessler method in a UV-2550 spectrophotometer (Shimadzu, Japan) [21]. *E. coli* concentrations were determined according to Chambonniere et al. (2023) [22], where removal efficiencies were expressed as percentage of removal and with logarithmic removal values (LVR) (log10•(C$_{\text{influent}}$/C$_{\text{effluent}}$). Dissolved phosphorous was measured following the orthophosphate determination method. In brief, 35 mL of sample (previously filtered through 0.45 nm filters and diluted if necessary) reacted with 10 mL of vanadate-molybdate solution (previously prepared from heptamolybdate and monovanate ammonia, at 25 and 1.25 mg L^{-1}, respectively) and makeup to 50 mL of total volume with distilled water, followed by a 10 min incubation time. The resulting solution was measured in a spectrophotometer at 470 nm (UV-2550 spectrophotometer (Shimadzu, Japan)). Particulate forms of phosphorus were not measured, due to the low TSS concentration measured on the secondary treated WW used as feedstock. Microalgae and cyanobacteria identification was performed by microscopic examination following the same procedure as Marín et al. (2021) [23].

Influent and effluent samples from the experimental system operating under steady-state conditions were systematically collected, along with purge biomass, using amber glass bottles during phases 1 and 2. Three samples per condition were used, time-spaced all along their respective steady state. Liquid samples were subsequently filtered using a two-step process: first through 0.7 μm glass fiber filters, followed by 0.45 μm nylon membrane filters. This procedure aimed to minimize microbial load and prevent sample degradation. Following filtration, all samples were stored at −20 °C until further processing. The analytical methods described by López-Serna et al. (2011) [24] and López-Serna et al. (2019) [25] were followed for CECs extraction (from liquid and solid phases) and quantitative analysis. In brief, a solid phase extraction was carried out on 100 mL of 0.45 μm filtered liquid samples fortified at 0.1 % Na_2EDTA and 1 μg/L of each internal standard compound using Oasis® HLB cartridges (60 mg, 3 cc; Waters Chromatography, Barcelona, Spain). Each cartridge was eluted twice with 3 mL per elution. The resulting solution was evaporated and then reconstituted in 1 mL of 0.1 % formic acid (FA) in H_2O/MeOH (95:5). The final solutions were analysed in a Sciex Exion UHPLC (Danaher, Washington DC, USA) coupled to a tandem triple-quadrupole Sciex 6500+ QqQ mass spectrometer. A Kinetex EVO C18 (2.1 mm × 50 mm, particle size 1.7 μm) reverse-phase column of Phenomenex (Danaher, Washington DC, USA) was used as stationary phase in the liquid chromatography (LC) section of the instrumental analysis. H_2O and MeOH-based solutions containing 0.1 % formic acid were run as mobile phases. In the mass spectroscopy (MS) section, an electrospray ionization interphase (ESI), working simultaneously both on positive and negative mode was set. Acquisition was carried out in Selected Reaction Monitoring (SRM) mode. The SRM transitions are shown in Supplementary Material. The resolution at q1 and q3 was stablished at units. For sample quantification, an 8-level standard addition calibration curve was built from 5 ng L^{-1} to 1×10^4 ng L^{-1}. Additionally, both calibration solutions and samples were fortified at 500 ng L^{-1} with internal standards.

The same methodology described above was followed for the quantitative determination of CECs in solid samples of settled biomass. However, these samples underwent previously a solid-liquid extraction consisting of a two-cycle ultrasound assisted extraction (UAE) of 0.3 g of lyophilized sample with a mixture methanol-H_2O (10:90). In addition, in contrast to the protocol described for liquids, internal standards were spiked in the lyophilized samples the day before the extraction was carried out. The 8-level regression curve for solids extended from 17 ng g^{-1} to 1333 ng g^{-1}.

2.6. Data treatment

NH_3 stripping was calculated based on a nitrogen balance of the system, attributing the net loss of nitrogen to this phenomenon. For this calculation, the following equation was used:

$$N_{stripping} = TN_{inf}Q_{inf} - TN_{efl}Q_{efl} - B_{purge}Q_{purge}{*}N_{biomass} \quad (1)$$

where $N_{stripping}$ corresponds to the mass of nitrogen loss per day by ammonia stripping (mg); TN_{inf} and TN_{efl} corresponds to the average total nitrogen concentration (mg L^{-1}) in the influent and the effluent of the system. Q_{inf}, Q_{efl} and Q_{purge} stands for the inlet, outlet and purged flow rates (L/d); B_{purge} stands for the biomass concentration on the purge (mg L^{-1}); and $N_{biomass}$ stands for the content of nitrogen mass on the biomass, set at 0.124 based on the standardized biomass formula for microalgae ($C_5H_7O_2N$).

The percentage of nitrogen lost by stripping was calculated based on the following equation:

$$N_{\%} = \frac{N_{stripping}}{TN_{inf}Q_{inf}} \times 100 \quad (2)$$

2.7. Economic analysis

A simplified techno-economic assessment was conducted for a tertiary/quaternary wastewater treatment system based on HRAPs to evaluate its economic implications. The system was designed to treat 1200 m^3 d^{-1} of municipal effluent from a population equivalent (p.e.) of 6000 and consisted of four HRAPs (HRT = 2 days, depth = 0.25 m, as in this study), requiring 9600 m^2 alongside an additional 20 % area designated for harvesting and auxiliary equipment. Continuous operation of the plant throughout all 365 days of the year was assumed.

Capital expenditure (CAPEX) included land costs, construction of HRAPs, and installation of harvesting units (Table 1, Supplementary Material). Land costs were estimated at 1 € m^{-2}, based on current agricultural land prices in Spain [26]. Although settling was used for biomass separation, the addition of flocculants is typically required to improve efficiency, which requires additional equipment such as a flocculation tank, settler, and pumping systems.

Operational expenditure (OPEX) included electricity, flocculants, maintenance and taxes and insurance. A breakdown is provided in Table 1, Supplementary Material. The electricity demand for this tertiary/quaternary treatment is primarily due to mixing (in the HRAP and flocculant tank) and water pumping. In this regard, an annual electricity consumption was estimated considering the electricity consumption in the HRAP (0.08 kWh per m^3) and a power consumption for harvesting and other processes of 0.1 kWh per m^3 [27,28]. Regarding harvesting, natural biopolymer-based flocculants, such as Tanfloc, offer a more sustainable alternative. An estimated dosage of 50 mg L^{-1} was assumed in these calculations, based on previous studies [29]. Labor costs have not been considered, as the plant will be operated by existing wastewater treatment plant staff.

Apart from the benefits of wastewater treatment, the main revenue for this system would be the valorisation of algal biomass as fertilizer. Assuming an average biomass productivity of 15 g m^{-2} d^{-1} in the HRAP, the estimated annual revenue would be 21,024 €.

3. Results and discussion

The following section presents the results obtained in this study under both HRT conditions. First, the environmental parameters that governed the system's performance are described. Subsequently, the results concerning key wastewater treatment parameters—such as carbon, nitrogen, and phosphorus removal, as well as the evolution of algal biomass growth—are presented. The HRAP's capacity for fecal pathogen removal is also reported. Finally, the last subsection describes the changes in the concentration of CECs in the system (in both the liquid phase and the biomass) for the two tested HRTs.

3.1. Environmental parameters

Temperature in the cultivation broth at steady state was of 27.4 ± 2.4 and 31.8 ± 1.5C in P1 and P2, respectively (Fig. 2A). The raise in temperature was caused by a rise in room temperature to 25 °C during P2 (plus the previous heat radiation of the LED system). The dissolved oxygen concentration in the system gradually increased from 2 mg L^{-1} to a mean value of 10.7 ± 2.2 mg L^{-1} in P1 and 10.6 ± 4.1 mg L^{-1} in P2 as a result of algal biomass growth. These dissolved oxygen concentrations were high enough to allow oxidation of both the residual organic matter and NH_4^+ present in the WWTP effluent (which typically require ≥2 mg L^{-1} O_2) [30]. Water evaporation accounted for 16.9 ± 8.0 % and

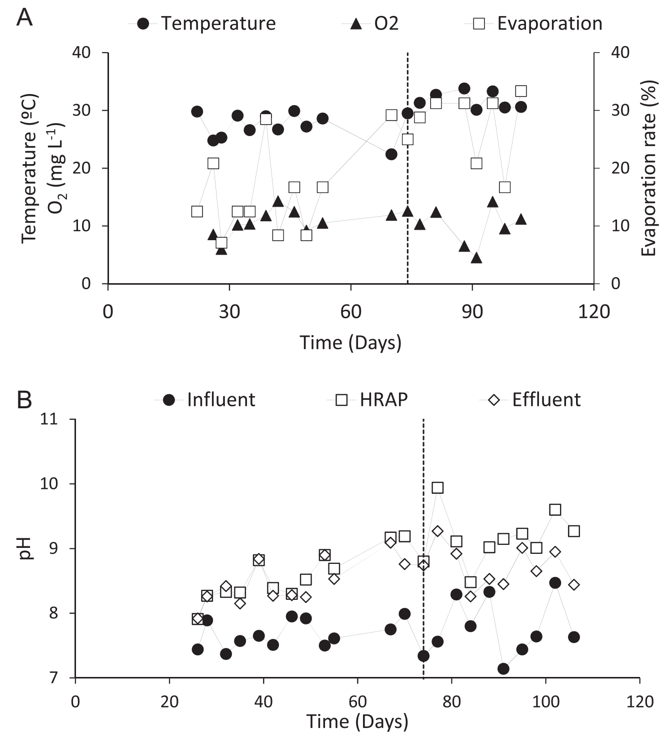

Fig. 2. Time course of A) temperature, dissolved oxygen concentration and evaporation rate, and B) pH in the cultivation broth of the HRAP. Dotted line indicates transition from P1 to P2.

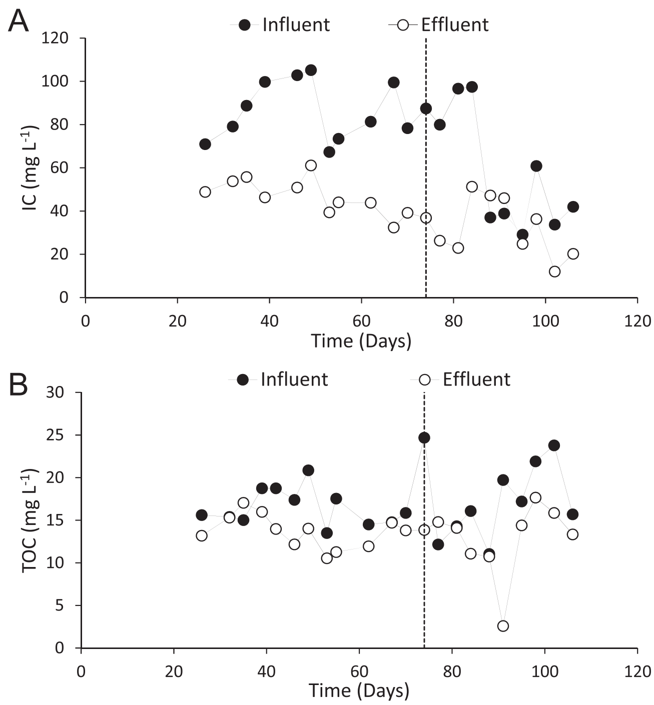

Fig. 3. Time course of the concentration of A) total organic carbon (TOC) and B) inorganic carbon (IC) during HRAP operation. Dotted line indicates transition from P1 to P2.

26.8 ± 6.3 % of the influent wastewater at HRT of 4 and 2 days, respectively. The pH of the HRAP culture broth increased from 7.77, a value similar to the feed (7.81), up to 8.4 ± 0.4 during P1 and 9.2 ± 0.4 during P2 (Fig. 2B). Thus, the cultivation broth of the HRAP underwent a gradual alkalinization process due to photosynthetic activity (uptake of CO_2) [13]. On the other hand, the pH of the feed remained constant throughout the process, with an average value of 7.75 ± 0.43. These environmental parameters ranged within typically reported values in previous researches [31].

3.2. *Carbon and nutrient removal*

The inlet IC concentration under steady state at an HRT of 4 days averaged 86.2 ± 13.1 mg L^{-1}, while the steady state outlet IC concentration at P1 accounted for 46.0 ± 8.4 mg L^{-1} (Fig. 3A). Similarly, the inlet and outlet concentrations at an HRT of 2 days averaged 57.3 ± 27.4 and 31.9 ± 13.8 mg L^{-1}, respectively. This corresponded to steady state removal efficiencies of 55.6 ± 10.8 and 50.5 ± 26.8 % at P1 and P2, respectively. IC plays a key role in the performance of HRAP, as it is the assimilable form of carbon by microalgae and nitrifying bacteria, and therefore, one of the major limiting factors of their growth [13]. IC may be assimilated by microalgae by direct CO_2 assimilation via plasmatic membrane; by converting HCO_3^- into CO_2 by the enzyme carbonic anhydrase; or by direct HCO_3^- transport through the plasmatic membrane [32]. A decrease in HRT implied a higher loading rate of IC, which in turn would allow a higher algal and oxygen productivity in the HRAP, provided that light intensity is not limiting [13]. The fact that the biomass grew in the photobioreactor under both HRTs demonstrated that the input of IC present in the influent was high enough to prevent growth limitation without the need of external carbon supply [33], also considering biomass settling and recirculation to the HRAP, which helps preventing biomass washout at the short HRTs herein tested. The HRT was also high enough to provide the HRAP with a high capacity to buffer the variations in the concentration of IC (present in the form of CO_2, HCO_3^- and CO_3^{2-}) in the feed. This fact was confirmed by the observed drop in the inlet IC concentration occurring between days 84 and 98 due to the active nitrification processes in the WWTP providing the treated domestic wastewater (Fig. 3A). Posadas et al. (2015) [30] observed a similar IC removal efficiency of 54 ± 19 % (55.6 ± 10.8 % in our study) during the treatment of 50:50 (*v*/v) fish farm and domestic wastewater in a 180 L HRAP operated at 7 days of HRT.

The inlet TOC concentrations under steady state for P1 and P2 averaged 17.1 ± 3.1 and 16.9 ± 4.3 mg L^{-1}, while the outlet concentrations averaged 13.0 ± 1.9 and 12.7 ± 4.4 mg L^{-1}, respectively. These concentrations corresponded to steady states removal efficiencies of 33.5 ± 12.2 % and 38.4 ± 26.4 % for P1 and P2, respectively. The fact that the removal efficiency remained similar between both operational periods suggests that a lower HRT can be used for organic carbon removal without significantly affecting the performance of the system regarding TOC. The slight increase in the TOC removal efficiency in P2 may be attributed to the higher growth of bacterial biomass, which assimilates some organics contained in the feed as a source of energy and carbon [13]. In this context, Posadas et al. (2015) [30] recorded a TOC removal efficiency of 65 ± 18 % at 7 days HRT in a HRAP treating a 50:50 (*v*/v) fish farm and domestic wastewater with 158 ± 67 mg TOC L^{-1} of inlet concentration.

On the other hand, the TN concentrations under steady state at the inlet flow averaged 36.9 ± 4.7 and 38.1 ± 11.2 mg L^{-1}, while the outlet values averaged 20.2 ± 4.5 and 22.4 ± 5.4 mg L^{-1}, for P1 and P2, respectively. These effluent concentrations were above the maximum of 10 mg L^{-1} required by current European regulations [9,34]. The removal of this aggregate pollutant in the HRAP remained constant throughout periods 1 and 2, with average removal values of 54.3 ± 11.6 % and 54.0 ± 7.3 %, respectively. In this context, the time course of the total nitrogen concentration (Fig. 4A) shows the capacity of the system to adapt to the varying nitrogen concentrations in the feed. The observed reduction in TN concentration in the HRAP was likely due to the assimilation of ammonium in the form of algal and bacterial biomass. Gómez-Serrano et al. (2015) [34] reported an effective tertiary treatment with microalgae in semi-continuous mode (with an effective HRT of 3.3 days), where negligible concentrations of nitrogen were reported

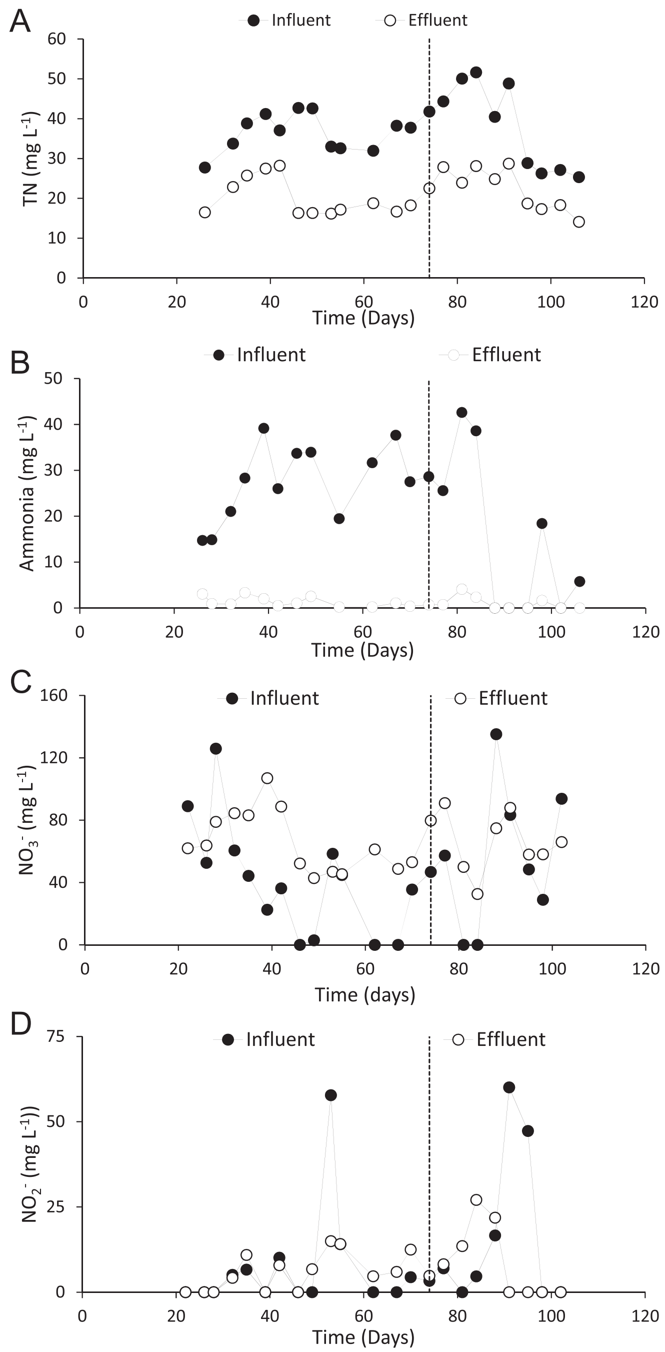

Fig. 4. Time course of the concentration of A) total nitrogen (TN), B) ammonia (NH_4^+), C) nitrate (NO_3^-) and D) nitrite (NO_2^-) during the HRAP operation. Dotted line indicates transition from P1 to P2.

in the effluent of the system at inlet concentrations of 17 mg L^{-1} (mostly in NH_4^+ form). Arbib et al. (2013) [35] obtained a TN removal efficiency of 65.1 ± 2.9 % operating a HRAP at 10 h of HRT during the tertiary treatment of domestic wastewater, with average TN inlet concentrations of 25.4 ± 1.7 mg L^{-1}.

Similarly, high total ammoniacal nitrogen concentrations of 27.5 ± 8.0 mg N L^{-1} were recorded in the influent of the HRAP in P1 as a result of the poor nitrification activity of the WWTP, while inlet ammoniacal nitrogen concentrations of 14.6 ± 17.4 mg N L^{-1} were observed in P2. The average concentrations in the effluent averaged 1.31 ± 1.1 and 0.98 ± 1.5 mg N L^{-1}, respectively. Thus, the HRAP was highly efficient removing NH_4^+ (Fig. 4B), with removal efficiencies of 95.7 ± 4.4 and 97.5 ± 3.2 % at HRTs of 4 and 2 days, respectively (Table 1). The removal of N-NH_4^+ by the algal-bacterial biomass was likely mediated by the uptake associated with the removal of residual organic matter and inorganic carbon, and by ammonium nitrification (the latter supported by the high dissolved oxygen and inorganic carbon concentrations). De Morais et al. (2022) [8] achieved a similar NH_4^+ removal efficiency of 95 % by using three interconnected 480 L photobioreactors at 24 h HRT as tertiary treatment system. In this context, Matamoros et al. (2016) [36] found a direct correlation between the removal of NH_4^+-N and the removal of CECs in wastewater. It is also important to highlight the potential loss of ammonium through stripping to the atmosphere. In this regard, a nitrogen balance was performed (Table 2), attributing the nitrogen loss in the system to ammonia stripping. A loss of 10.8 ± 21.5 % was estimated in P1 and 27.0 ± 33.0 % in P2. The increase in nitrogen loss observed at 2 days of HRT was consistent with the higher temperature and higher pH recorded in this operational phase, despite the shorter exposure time to the atmosphere resulting from the reduced HRT.

The nitrate concentration (Fig. 4C) in the effluent of the HRAP (66.9 ± 20.0 mg L^{-1}) during P1 was, on average, higher than the concentration in the feed (48.6 ± 30.6 mg L^{-1}), which suggested the occurrence of an active nitrification in the HRAP at the HRT of 4 days. However, the active nitrification in the full-scale activated sludge WWTP providing the treated water in P2 resulted in nitrate concentrations slightly higher in the influent (74.5 ± 37.9 mg L^{-1}) than in the effluent (64.8 ± 19.5 mg L^{-1}) (Fig. 4C). Thus, the nitrate removal efficiencies averaged −15.5 % (= generation of nitrate from ammonium oxidation) and 22.08 % in P1 and P2, respectively. On the other hand, nitrite concentrations (Fig. 4D) averaged 14.5 ± 19.5 and 27.1 ± 25.1 mg L^{-1} in the influent, while 8.69 ± 4.1 and 17.7 ± 8.4 mg L^{-1} were detected in the effluent during P1 and P2, respectively. In this context, the removal efficiencies of nitrite under steady state were 50.0 % and 41.6 % in P1 and P2, respectively. Overall, the high nitrate concentrations observed in the HRAP effluent could indicate a nitrogen input exceeding the treatment capacity of the photosynthetic system regardless of the HRTs tested. The near complete removal of NH_4^+ appears to be the result of an increased activity of the nitrifying bacteria oxidizing this compound to NO_2^- and NO_3^- [10]. At this point it should be highlighted that ammonia nitrification is a mechanism of ammonia removal, which do not entail nitrogen removal.

The influent phosphate concentrations under steady state averaged 2.55 ± 2.9 and 1.22 ± 0.63 mg L^{-1} for P1 and P2, respectively, while the effluent concentrations averaged 1.19 ± 0.6 and 0.59 ± 0.3 mg L^{-1}, respectively (Fig. 5). Hence, the phosphate removal efficiency remained relatively constant throughout the process (42.6 ± 21.6 and 39.3 ± 35.8, in P1 and P2, respectively). The concentration peak in the feed during days 35 and 39 in phase 1, reaching concentrations up to 10 times higher than the average, was likely due to a punctual malfunction of the WWTP. Excluding this phosphate concentration peak, the effluent phosphate concentrations remained below 1 mg L^{-1}, as required by current European regulations [9,34]. Phosphate removal in HRAP systems occurs by precipitation induced by the raise of pH (due to photosynthetic activity), as well as by microalgae assimilation in the form of biomass and accumulation as polyphosphates (the latter mechanism known as luxury uptake) [13]. It is worth mentioning the lower phosphate concentration measured in the influent at P2, along with the higher standard deviation observed for TN at this condition. Based on the availability of inorganic and organic carbon in the cultivation, the larger variation in nitrogen removals recorded in P2 might be attributed to a lower N:P ratio. Other authors, such as Sukačová et al. (2015) [37] showed a similar phosphate removal efficiency of up to 41 ± 11 % using a microalgae biofilm photobioreactor illuminated with real sunlight in a tertiary treatment process with an inlet concentration of 2.9 ± 0.1 mg P L^{-1}. Gómez-Serrano et al. (2015) [34] achieved negligible concentrations of phosphate in the effluent of an effective tertiary treatment with microalgae in semi-continuous mode (with an effective HRT of 3.3 days)

Table 2

Nitrogen balance calculated in the system for both periods, based on the nitrogen contained on the influent, the effluent and biomass purged per day. The nitrogen missing in the balance was attributed to the stripping of ammonia to the atmosphere.

	Purge ($g_{biomass}$)	N Influent (mg)	N Efluent (mg)	N Biomass (mg)	N stripping (mg)	Stripping (%)
Period 1[a]	0.31 ± 0.09	88.5 ± 11.3	39.7 ± 9.9	39.1 ± 11.3	9.58 ± 19.0	10.8 ± 21.5
Period 2	0.46 ± 0.15	182.9 ± 53.6	78.8 ± 20.1	56.5 ± 12.3	49.3 ± 60.3	27.0 ± 33.0

[a] Period 1 and Period 2 correspond to HRT values of 4 and 2 days, respectively.

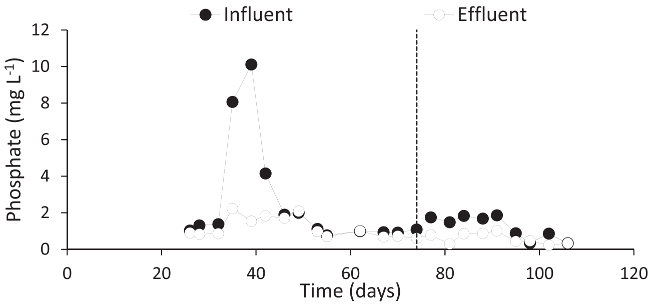

Fig. 5. Time course of the concentration of phosphate (PO_4^-) during HRAP operation. Dotted line indicates transition from P1 to P2.

at an inlet concentration of 10 mg L^{-1}. Arbib et al. (2013) [35] reported a phosphate removal efficiency of 58.8 ± 1.2 % in a HRAP operating at 10 h HRT during tertiary treatment with average inlet concentrations of 2.03 ± 0.13 mg P L^{-1}. In this regard, Velasquez-Orta et al. (2024) [38] performed a meta-analysis of different HRAPs at pilot scale, observing an average removal of 50 % in PO_4^{3-} together with 90 % in NH_4^+, which are similar values than those of our study.

The biomass in the HRAP grew gradually over time and was maintained at 1 g/L via periodic biomass wasting from the bottom of the settler (Fig. 6). In this regard, once the HRAP reached this target biomass concentration, a 0.1 L purge was carried out each sampling day, accounting for 1.11 ± 0.33 and 1.59 ± 0.54 g of total biomass removed per sampling event in P1 and P2, respectively. Thus, average biomass concentrations in the HRAP of 0.9 ± 0.2 and 1.0 ± 0.3 mg TSS L^{-1} were recorded during P1 and P2, respectively (with a VSS/TSS ratio of 0.73 ± 0.09). Moreover, the biomass concentration in the effluent (after settling) averaged 10 mg TSS L^{-1}, which was lower than the 35 mg L^{-1} required by current European regulations [9]. The high biomass growth rate, together with the high aggregation and precipitation capacity of the algal-bacterial biomass, allowed the HRAP to be operated at HRTs as low as 2 days (which typically entail algal washout in HRAP without biomass recirculation). Under this operational strategy, the solid retention time (SRT) set during P1 and P2 accounted for 8.0 ± 3.0 and 6.5 ± 2.8 days, respectively. The higher SRT values compared to the HRTs (in both conditions) allowed to avoid the washout of microalgae and nitrifiers despite the low HRTs tested [39]. These values of HRT at the prevailing temperatures of the culture broth guaranteed the establishment of a stable algae and nitrifying population.

The capacity of the HRAP to eliminate pathogens during tertiary treatment was assessed using *E. coli* as indicator species, a highly widespread fecal bacterium in domestic wastewater. The average concentration of *E. coli* in the effluent under steady state was 0.7 ± 1.4 and 3.8 ± 5.2 CFU mL^{-1} during P1 and P2, respectively (Fig. 7). Influent *E. coli* concentrations of 28.6 ± 15.2 CFU mL^{-1} were recorded during P1, while the *E. coli* concentration increased up to 600 CFU mL^{-1} in P2. Despite the rapid rise in *E. coli* concentration in the feed at the end of P2, the HRAP was highly effective at eliminating this pathogen, with an average removal efficiency of 97.4 ± 4.5 % and 99.0 ± 0.4 % at P1 and P2, respectively. These removal efficiencies corresponded to logarithmic removal values (LRV) of 1.59 and 1.75, respectively. The extended exposure to light (visible and UV), the predatory effect (by viruses, protozoa and other organism) and the toxicity for pathogens induced by the high pH and O_2 concentration were likely the main mechanisms for this effective pathogen removal [7,31]. The effluent *E. coli* concentrations recorded in this experiment were below the maximum limit for the reuse of water for agriculture imposed in Spain (200 CFU/ 100 in 50 % of samples or 1000 CFU/100 mL in 90 % of the samples measured) [40]. Ruas et al. (2018) [41] obtained similar removal efficiencies in an HRAP during secondary domestic wastewater treatment at a HRT of 5 days: 98.6 % of *E. coli*, 99.7 % of *Enterococci* sp. and 97.4 % of *Pseudomonas aeruginosa*, with an average removal efficiency of total coliforms of 88.7 %. Buchanan et al. (2018) [42] obtained a similar LRV of 1.75 in an HRAP treating a septic tank wastewater at 4 days HRT. Finally, Chambonniere et al. (2020) [43] obtained an average *E. coli* LRV of 1.77 ± 0.54 in two 0.83 m^3 HRAPs fed with domestic wastewater at 10.3 days of HRT.

Overall, the application of a tertiary treatment based on microalgae-bacteria associations supported an effective removal of nutrients, carbon and pathogens. The use of microalgae for wastewater treatment has consistently reported in literature for secondary treatment [44,45], but also as an efficient tertiary treatment platform [32]. Here, the decrease in HRT from 4 to 2 days did not exert a significant impact on the performance of the HRAP, which was likely due to the effective retention of algal-bacterial biomass mediated by the settling and recirculation. Indeed, the observed differences in process performance were more related to variations in the composition of the influent wastewater than to the increase in wastewater load.

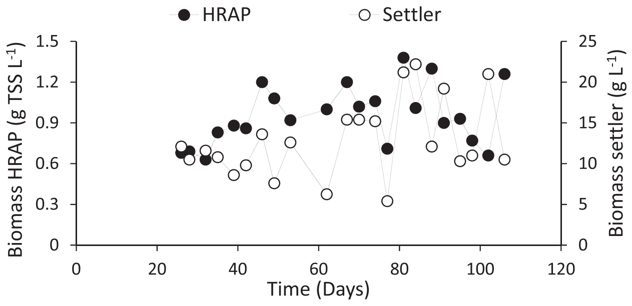

Fig. 6. Time course of the concentration of biomass in the HRAP and settler measured as TSS. Dotted line indicates transition from P1 to P2. VSS/TSS ratio was 0.73 ± 0.09.

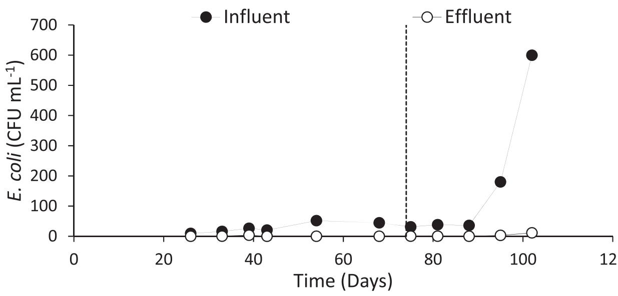

Fig. 7. Time course of the concentration of *Escherichia coli* during the HRAP operation. Dotted line indicates transition from P1 to P2.

3.3. CECs removal

For a significant number of the target CECs compounds analysed in the effluent of the WWTP, herein used as influent in the HRAP for tertiary and quaternary treatment, concentrations below the limits of detection (MDL) and quantification (MQL) were observed (Table 3). Therefore, the discussion was herein elaborated in terms of detected or non-detected compounds rather than concentrations and removals for each of the individual 44 CECs analysed. At an HRT of 4 days, the HRAP was able to remove the following 9 CECs below their limits of quantification: nalidixic acid, sulfamethoxazole, clarithromycin, apramycin, naproxen, acetaminophen, atorvastatin, propranolol and atenolol. On the other hand, at 2 days of HRT, the HRAP removed the following 5 CECs under the limit of quantification: atenolol, acetaminophen, clofibric acid, progesterone and methylparaben. These results confirmed the high efficiency of HRAPs for the removal of emerging pollutants as a post-treatment or polishing step [46,47].

For ofloxacin (P1), tiamulin (P1), naproxen (P2), salicylic acid (P1), atorvastatin (P2), carbamazepine (P1 & P2), DEET (P1 & P2), atrazine (P2), 4-hidroxybenzoic acid (P1) and caffeine (P2), similar concentrations in the influent and effluent of the HRAP were observed (Table 3). Unexpectedly, higher concentrations of CECs were found in the effluent for some of these compounds. This may be due to the delays caused by the high operational HRTs (4 and 2 days in period 1 and 2, respectively) and the inherent fluctuations in the inlet CECs concentrations in the real secondary effluent treated. In addition, the high evaporation rates (ranging from 7.1 to 29.2 % and from 16.7 to 33.4 % of the influent flowrate at P1 and P2, respectively) inherently caused an increase in the concentration of all CECs in the treated WW.

Some CECs with unusually high differences in the concentration between periods were identified (Table 3). For instance, salicylic acid was present at much higher concentration in the inlet in period 2 (6446 ng L^{-1}) than in period 1 (34 ng L^{-1}), although medium to good removal efficiencies were recorded in both cases (19.7 and 71.6 % at P1 and P2, respectively). Additionally, diclofenac was found at the HRAP outlet at a concentration of 2788 ng L^{-1} in period 1 in comparison to 361 ng L^{-1} in period 2 (average values of 461 and 181 ng L^{-1} were recorded at the inlet in P1 and P2, respectively), which entails one order of magnitude of

Table 3
Concentrations of contaminants of emerging concern measured in the influent, effluent and biomass during the wastewater treatment in an algal-bacterial photobioreactor.

		Period 1[a]			Period 2		
		Effluent (ng L^{-1})	Influent (ng L^{-1})	Biomass (ng g^{-1})	Effluent (ng L^{-1})	Influent (ng L^{-1})	Biomass (ng g^{-1})
Antibiotics	Penicillin G	<MDL	<MQL	<MQL	<MQL	<MQL	<MDL
	Doxycycline	<MQL	<MDL	<MQL	<MDL	<MDL	<MQL
	Tetracycline	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Ciprofloxacin	243	105	55	242	150	53
	Ofloxacin	90	94	33	108	30	26
	Levofloxacin	66	216	24	160	74	18
	Norfloxacin	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL
	Nalidixic acid	<MQL	9	<MQL	<MQL	<MQL	<MDL
	Sulfadimidine	<MDL	<MDL	<MDL	<MQL	<MQL	<MQL
	Sulfadiazine	<MQL	<MDL	<MDL	<MQL	<MQL	<MQL
	Sulfamethoxazole	<MQL	126	<MDL	<MQL	<MDL	<MDL
	Sulfamethizole	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL
	Sulfathiazole	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Sulfapyridine	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Tylosin	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Erythromycin	<MDL	<MDL	<MDL	<MQL	<MQL	<MDL
	Clarithromycin	<MDL	125	<MQL	73	<MQL	<MQL
	Apramycin	<MDL	64	<MDL	<MDL	<MDL	<MDL
	Tiamulin	3	16	<MDL	<MQL	<MDL	5
	Florfenicol	<MDL	<MQL	<MQL	<MDL	<MDL	<MQL
	Trimethoprim	<MDL	<MQL	217	<MDL	<MDL	<MQL
Anti-inflammatories	Dexamethasone	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Diclofenac	2788	461	<MQL	361	181	590
	Naproxen	<MQL	255	<MQL	94	59	<MQL
	Ibuprofen	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Acetaminophen	<MQL	63	174	<MQL	15	137
	Salicylic acid	53	34	32	1849	6446	17
Lipid regulators	Clofibric acid	<MDL	<MDL	<MDL	<MDL	28	<MDL
	Atorvastatin	<MQL	64	<MDL	25	12	<MDL
	Gemfibrozil	182	243	<MDL	172	45	<MDL
Beta-blockers	Propranolol	<MDL	27	<MQL	13	<MQL	28
	Atenolol	<MDL	175	<MDL	<MDL	184	<MQL
Psychiatric drugs	Carbamazepine	21	13	4	20	25	3
Anti-parasitics	Metronidazole	<MQL	42	<MDL	<MQL	<MQL	<MDL
	Fenbendazole	<MDL	<MQL	<MQL	<MDL	<MDL	<MQL
Hormones	Progesterone	<MQL	<MQL	<MQL	<MQL	42	<MQL
Insect repellents	DEET	106	80	18	103	168	27
Pesticides	Atrazine	<MDL	<MQL	<MQL	2	2	13
Cosmetic preservatives	Methylparaben	<MDL	<MQL	120	<MDL	121	74
	Ethylparaben	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL
	Propylparaben	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL
	4-hidroxybenzoic acid	52	40	33	490	170	18
Stimulants	Caffeine	320	<MDL	13	135	90	7
Anti-pruritics	Crotamiton	<MQL	<MQL	<MQL	<MQL	<MQL	<MDL
Total concentration		3924	2252	723	3847	7842	1016

Note: <MDL for concentrations below the detection limit. <MQL for samples below the quantification limit.

[a] Period 1 and Period 2 correspond to HRT values of 4 and 2 days, respectively.

difference. HRAPs have shown high removal efficiencies of diclofenac (identified as a high priority compound by the EU under the Water Framework directive) during domestic wastewater treatment [48]. In this context, Escapa et al. (2016) [49] demonstrated the ability of *Chlorella vulgaris* to tolerate high concentrations of diclofenac, reaching up to 79 % removal efficiencies in a bubbling column photobioreactor treating synthetic culture medium (25 mg L^{-1} of inlet diclofenac) in semicontinuous mode with an effective HRT of 3.3 days.

Overall, excluding diclofenac on period 1, lower total CEC concentrations in the effluent compared to the influent of the HRAP were recorded in both periods, with similar removal efficiencies in liquid phase of 57.6 ± 4.0 % and 63.7 ± 3.1 % for period 1 and 2, respectively. In this regard, Hom-Diaz et al. (2017) [50] observed pharmaceutical removal efficiencies higher than 48 % in a microalgal photobioreactor treated toilet WW at 12 and 8 h of HRT. Similarly, Matamoros et al. (2015) [51] observed no statistical difference in CECs removal at 8 and 4 days of HRT in a HRAP treating urban wastewater in summer, where environmental conditions may be comparable to the temperatures prevailing in the HRAP of this work (28.3 ± 2.4 °C). Comparing our tertiary treatment with a non-HRAP based treatment, Chen et al. (2024) [6] analysed the efficiency of 2 activated sludge WWTPs following a tertiary treatment based on a high efficiency sedimentation tank (HEST) followed by a denitrification deep bed filter (DDBF). In this study, the tertiary treatment removed 10.2 and 55.8 % of the CECs from the secondary effluents, accounting for 2.93 and 2.36 % of the total WWTP CECs removal. Similar to our study, the tertiary system was efficient at removing certain CECs such as paracetamol, sulfamethoxazole etc… while it was totally inefficient for other CECs, even increasing their concentration as in the particular case of carbamazepine.

In addition, the biomass samples analysed also exhibited rather fluctuating CEC concentrations (Table 3). Many of the target compounds were present below the detection and quantification threshold. Most of those CEC quantified in the biomass belonged to the groups such as fluoroquinolones (ciprofloxacin, ofloxacin and levofloxacin), with 112 (P1) and 107 (P2) ng g^{-1} in total; or anti-inflammatories (diclofenac, naproxen, acetaminophen and salicylic acid), with 204 (P1) and 744 (P2) ng g^{-1} in total. This groups are characterized by their high partition coefficients (logP), which facilitates their transition from the liquid to the solid phase of the biomass [52,53].

CEC concentrations in the biomass were also relevant all along the process, indicating that bioaccumulation and bio-adsorption in the algal-bacterial biomass were remarkable during tertiary and quaternary treatment in the HRAP. Interestingly, the HRT impacted this CEC accumulation in the biomass. Hence, lower HRTs resulted in higher CECs concentrations in the algal-bacterial biomass (723 ng L^{-1} at P1, 1016 ng L^{-1} at P2), and consequently higher removal efficiency mediated by the increasing biomass production rates at lower HRTs [51]. This CEC concentration in biomass allows us to acknowledge what kind of degradation processes are more important for each CEC and HRT condition, distinguishing between bioadsorption/bioaccumulation processes and biodegradation/biophotolysis/hydrolysis ones. The increase in the CECs concentration in the biomass at lower HRT, while supporting similar overall CECs removals between the two operational conditions, suggests that bioadsorption/bioaccumulation mechanisms act faster than biodegradation mechanisms. Thus, it is likely that the CECs retained in the biomass via bioadsorption could be more susceptible to removal via biophotolysis and biohydrolysis due to their higher exposure to radiation and environmental conditions, while those retained by bioaccumulation could be more prone to removal by biodegradation since they are already inside the microalgae/bacterial cells. Unfortunately, it was not possible to determine the extent of each specific mechanism with the methodology used in this study.

It is noteworthy that the MDL and MQL were, in general, higher in the effluent than in the influent. This entailed that some CECs could not be detected or quantified in the effluent but were quantified in the influent. Typically, wastewater treatment processes eliminate interfering compounds, resulting in simplified matrices that entail a lower signal-to-noise ratio and, therefore, lower MDL and MQL at the effluent of the system in comparison to the influent. In this particular study, the effluent of the microalgae-based treatment system likely presented interfering compounds, such as photosynthetic pigments (chlorophylls, carotenoids, etc…) as well as multiple organic metabolites, which increased the signal to noise ratio and, thus, the MDL and MQL [19,54]. This could explain the higher limits of detection and quantification estimated for many CECs at the effluent. The MDL and MQL for the target compounds are given in Supplementary material.

Overall, the proposed system effectively removed approximately 60 % of the CECs present in the secondary-treated wastewater, with no significant differences observed between the applied HRTs. However, the removal behavior of individual CECs varied according to their physicochemical properties, with some compounds showing high removal efficiencies, while others were barely affected by the tertiary/quaternary treatment. The accumulation patterns of each CEC in the biomass provided insights into the predominant removal mechanisms for specific compounds, although a comprehensive understanding of these processes cannot be achieved with the methodology applied in this study. While the use of a generalized approach allowed for an overall assessment of CEC removal, a more targeted methodology is required to elucidate the behavior of individual CECs (or groups of CECs) under the proposed tertiary treatment conditions.

3.4. Sustainability and economic analysis

This simplified economic evaluation of the HRAP-based tertiary/quaternary treatment system provides insights into its cost structure and operational feasibility. The total capital expenditure was mainly driven by construction costs of the ponds and harvesting units, while operational costs were dominated by electricity consumption and flocculants usage. The capital investment required for the system (85,943 €) is competitive when compared to membrane-based tertiary treatments, which typically involve higher infrastructure costs (approximately 400 € pe^{-1}) vs 71.62 € pe^{-1} in the case of microalgae-based systems [55]. Operational costs (45,723 € per year, equivalent to 0.104 € m^{-3}) are lower also compared to other tertiary treatment methods such as membrane filtration, photo-Fenton processes, or constructed wetlands (0.4–1.0 € m^{-3}). However, they are higher than those associated with simpler disinfection techniques like chlorination or ozonation, which typically cost around 0.004 € m^{-3} and 0.03 € m^{-3}, respectively [56].

Domestic wastewater treatment is typically performed using activated sludge processes, with operational costs typically ranging from 0.17 to 0.75 € per cubic meter [57,58]. Therefore, the combination of conventional secondary activated sludge treatment with algal-based tertiary/quaternary processes results in a higher overall OPEX (0.27–0.85 € m^{-3}). Nonetheless, this approach offers added value through enhanced nutrient removal and the potential for biomass valorisation, with an estimated annual biomass productivity equivalent to 21,024 € (0.048 € m^{-3}).

In terms of environmental impact, the installation of a HRAP system for tertiary/quaternary treatment would imply an indirect increase in CO_2 equivalent emissions due to the energy consumption for culture mixing. For the aforementioned reference scenario, the implementation of a microalgal-based system for tertiary/quaternary treatment would result in an increase in CO_2 emissions due to electricity consumption of 19.8 ton-eq per year, assuming a CO_2 footprint of 0.174 kg equivalent per kWh. Furthermore, it has been proven that this type of plant might emit significant quantities of nitrous oxide (N_2O) into the atmosphere, representing an emission with a high greenhouse effect (298 kg CO_2 equivalent kg N_2O^{-1}) [59]. In the reference case, N_2O emissions from the HRAP would represent 779 kg CO_2-eq y^{-1} considering the empirical ratio of 0.05 mg N-N_2O g N $input^{-1}$ [60]. Nevertheless, direct CO_2 reductions of 96.2 ton-eq per year would occur in this process as a result of photoautotrophic microalgal growth, considering a biomass production of 52.56 ton y^{-1} and 1.83 g of CO_2 consumed for each gram of algal biomass. As a result, this process will contribute to decrease the carbon footprint of the wastewater system. There is also a loss of water in the process, due to biomass harvesting and, more significantly, evaporation from the HRAPs, given the large surface area required. Although approximately 27 % of the influent wastewater was lost at an HRT of 2 days, the mixing system used in this study was oversized and not representative of a large-scale system. In this regard, considering an average evaporation loss of 9 L d^{-1} m^{-2} in temperate climates, the total annual water loss would be approximately 31,536 m^3, representing 7.2 % of the influent wastewater [61]. The environmental impact of the plant must be taken into account in the evaluation of the balance between the ecological damage to be avoided by the pre-discharge wastewater treatment compared to the environmental impact involved in the implementation of the treatment plant. Here, it is important to emphasise the high CECs removal capacity of the HRAP and the benefits associated to their removal [5,11]. Overall, microalgae-based tertiary treatment is a viable option, especially in regions with land availability and strong environmental regulations on nutrient discharge.

In conclusion, the implementation of a tertiary/quaternary treatment entails an increase in both economic and environmental costs, which must be carefully considered when assessing the overall viability of the process. These additional costs should be weighed against the improvements achieved in the removal of nutrients, pathogens, and CECs, as discussed in the previous section. The potential valorization of the algal biomass produced may partially offset the additional expenses associated with the integration of this advanced treatment stage into domestic wastewater management.

3.5. *Limitations of this study*

Although the present study provides valuable information on the tertiary/quaternary wastewater treatment potential of microalgae, it should be noted that the proposed experimental design has certain limitations. On the one hand, the concentrations of the pollutants at the inlet of the system underwent large variations throughout the operation of the process as a result of fluctuations in the treatment performance of the preceding activated sludge process. This implied a lack of stability in many of the parameters measured in the system, which makes it difficult to draw certain conclusions from the data recorded, especially with regard to the legal limits that must be fulfilled when discharging water into the environment. On the other hand, although the analysis of the 44 CECs targeted in this research has simultaneously allowed us to observe their interaction when they are removed from the system, the application of a general analytical method for all these compounds has implied MQLs and MDLs with very high values given the low concentrations of many of the CECs analysed. Therefore, the subsequent application of more specific methods for groups of CECs could be relevant in order to know the specific concentrations of these pollutants.

4. Conclusions

The HRAP configuration herein tested was effective at removing contaminants during tertiary and quaternary treatment of domestic WW at short HRT (4 and 2 days). Biomass settling and recirculation prevented biomass washout at 4 and 2 days of HRT. The tested HRTs did not exert significant differences in the capability of the HRAP to remove contaminants from treated wastewater. The system was able to maintain phosphate, *E. coli* and biomass concentrations in the effluent below EU discharge limits, while TN concentrations exceeded such limits, mainly in the form of nitrite ($P1 = 8.69$ and $P2 = 17.7$ mg L^{-1}) and nitrate ($P1 = 66.9$ and $P2 = 64.8$ mg L^{-1}). In this context, nearly all inlet NH_4^+-N (>95 %) was removed in the clarified effluent, mediated by an effective assimilation in the form of algal biomass and primarily by nitrification.

The removal of CECs was influenced by the inherent variations of their concentration in the influent of the HRAP, where the tested HRTs did not substantially impact their removal. Overall, the HRAP supported similar CECs removal efficiencies of approximately 60 %, despite the concentrative effect caused by the high evaporation rates observed during the operation. In this regard, 9 compounds were removed below their MQL in P1 and 5 compounds in P2. On the other hand, the accumulation of CECs in the biomass increased at increasing wastewater loading rates, which provide interesting insights into the microalgae mechanism for CEC removal (i.e. bioadsorption/bioaccumulation/biodegradation). Despite the high removal efficiencies recorded in this study, further research in needed to optimize HRAP performance during quaternary treatment.

CRediT authorship contribution statement

Lois Regueira-Marcos: Visualization, Methodology, Investigation, Formal analysis, Conceptualization, Writing – review & editing, Writing – original draft. **Rebeca López-Serna:** Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization, Writing – review & editing. **Octavio García-Depraect:** Visualization, Writing – review & editing. **Maria del Rosario Rodero:** Visualization, Validation, Supervision, Methodology, Formal analysis, Writing – review & editing. **Ana Alcoceba:** Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Raúl Muñoz:** Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization, Writing – review & editing.

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Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2025.104239.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available since the project is financed by a private company, but are available from the corresponding author on reasonable request.

References

[1] K.J. Chae, J. Kang, Estimating the energy independence of a municipal wastewater treatment plant incorporating green energy resources, Energy Convers. Manag. 75 (2013) 664–672, https://doi.org/10.1016/J.ENCONMAN.2013.08.028.

[2] M.C. Ozturk, F. Martin Serrat, F. Teymour, Optimization of aeration profiles in the activated sludge process, Chem. Eng. Sci. 139 (2016) 1–14, https://doi.org/10.1016/J.CES.2015.09.007.

[3] J. Jimenez, M. Miller, C. Bott, S. Murthy, H. De Clippeleir, B. Wett, High-rate activated sludge system for carbon management – evaluation of crucial process mechanisms and design parameters, Water Res. 87 (2015) 476–482, https://doi.org/10.1016/J.WATRES.2015.07.032.

[4] M. Petrović, S. Gonzalez, D. Barceló, Analysis and removal of emerging contaminants in wastewater and drinking water, TrAC Trends Anal. Chem. 22 (2003) 685–696, https://doi.org/10.1016/S0165-9936(03)01105-1.

[5] R. dos S. Grignet, M.G.A. Barros, A.A.S. Panatta, S.P.F. Bernal, J.R. Ottoni, M.R.Z. Passarini, C. da C. S. Gonçalves, Medicines as an emergent contaminant: the review of microbial biodegration potential, folia. Microbiol. (Praha) 67 (2022) 157–174. doi:https://doi.org/10.1007/s12223-021-00941-6.

[6] T. Chen, Q. Zeng, M. Cao, L. Zhang, B. Adyari, C. Ma, K. Wang, D. Gao, A. Hu, Q. Sun, C.P. Yu, Fate of contaminants of emerging concern in two wastewater treatment plants after retrofitting tertiary treatment for reduction of nitrogen discharge, Environ. Res. 249 (2024), https://doi.org/10.1016/j.envres.2024.118344.

[7] P. Chambonniere, J. Bronlund, B. Guieysse, Pathogen removal in high-rate algae pond: state of the art and opportunities, J. Appl. Phycol. (2021), https://doi.org/10.1007/s10811-020-02354-3.

[8] E.G. de Morais, J.C. Amaro Marques, P.R. Cerqueira, C. Dimas, V.S. Sousa, N. Gomes, M. Ribau Teixeira, L.M. Nunes, J. Varela, L. Barreira, Tertiary urban wastewater treatment with microalgae natural consortia in novel pilot photobioreactors, J. Clean. Prod. 378 (2022) 134521, https://doi.org/10.1016/J.JCLEPRO.2022.134521.

[9] R. Whitton, F. Ometto, M. Pidou, P. Jarvis, R. Villa, B. Jefferson, Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, environ, Technol. Rev. 4 (2015) 133–148, https://doi.org/10.1080/21622515.2015.1105308.

[10] S. Rani, R. Chowdhury, W. Tao, L. Nedbalova, Microalga-mediated tertiary treatment of municipal wastewater: removal of nutrients and pathogens, Sustainability 13 (2021), https://doi.org/10.3390/su13179554.

[11] D.L. Sutherland, P.J. Ralph, Microalgal bioremediation of emerging contaminants - opportunities and challenges, Water Res. 164 (2019) 114921, https://doi.org/10.1016/J.WATRES.2019.114921.

[12] K. Li, Q. Liu, F. Fang, R. Luo, Q. Lu, W. Zhou, S. Huo, P. Cheng, J. Liu, M. Addy, P. Chen, D. Chen, R. Ruan, Microalgae-based wastewater treatment for nutrients recovery: a review, Bioresour. Technol. 291 (2019) 121934, https://doi.org/10.1016/J.BIORTECH.2019.121934.

[13] C. Alcántara, C. Fernández, P.A. García-Encina, R. Muñoz, Mixotrophic metabolism of Chlorella sorokiniana and algal-bacterial consortia under extended dark-light periods and nutrient starvation, Appl. Microbiol. Biotechnol. 99 (2015) 2393–2404, https://doi.org/10.1007/s00253-014-6125-5.

[14] F. Hussain, S.Z. Shah, H. Ahmad, S.A. Abubshait, H.A. Abubshait, A. Laref, A. Manikandan, H.S. Kusuma, M. Iqbal, Microalgae an ecofriendly and sustainable wastewater treatment option: biomass application in biofuel and bio-fertilizer production. A review, renew, Sustain. Energy Rev. 137 (2021) 110603, https://doi.org/10.1016/J.RSER.2020.110603.

[15] S. Mehariya, R.K. Goswami, P. Verma, R. Lavecchia, A. Zuorro, Integrated approach for wastewater treatment and biofuel production in microalgae biorefineries, Energies (Basel) 14 (2021), https://doi.org/10.3390/en14082282.

[16] R.J. Davies-Colley, A.M. Donnison, D.J. Speed, C.M. Ross, J.W. Nagels, Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: interactions of environmental factors with sunlight, Water Res. 33 (1999) 1220–1230, https://doi.org/10.1016/S0043-1354(98)00321-2.

[17] R. Gondi, S. Kavitha, R. Yukesh Kannah, O. Parthiba Karthikeyan, G. Kumar, V. Kumar Tyagi, J. Rajesh Banu, Algal-based system for removal of emerging pollutants from wastewater: a review, Bioresour. Technol. 344 (2022) 126245, https://doi.org/10.1016/J.BIORTECH.2021.126245.

[18] AEMPS (2011). Datos de ventas de antimicrobianos en España en el año 2009. Ministerio de sanidad, política social e igualdad. https://www.aemps.gob.es/informa/notasInformativas/medicamentosVeterinarios/2011/docs/ventas-antimicrobianos_Espana-2009.pdf.

[19] J.Q. Jiang, Z. Zhou, V.K. Sharma, Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water — a review from global views, Microchem. J. 110 (2013) 292–300, https://doi.org/10.1016/J.MICROC.2013.04.014.

[20] E.A. D., L.S. Clesceri, E.W. Rice, A.E. Greenberg, Standard methods for the examination of water and wastewater, (2008). http://tvhdh.vnio.org.vn:8080/xmlui/handle/123456789/14732 (accessed January 22, 2025).

[21] E. Posadas, P.A. García-Encina, A. Soltau, A. Domínguez, I. Díaz, R. Muñoz, Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors, Bioresour. Technol. 139 (2013) 50–58, https://doi.org/10.1016/J.BIORTECH.2013.04.008.

[22] P. Chambonniere, M. Plouviez, B. Guieysse, Modelling Escherichia coli removal during domestic wastewater treatment in outdoor pilot scale high rate algal ponds, J. Appl. Phycol. 35 (2023) 1203–1212, https://doi.org/10.1007/s10811-023-02947-8.

[23] D. Marín, A.A. Carmona-Martínez, S. Blanco, R. Lebrero, R. Muñoz, Innovative operational strategies in photosynthetic biogas upgrading in an outdoors pilot scale algal-bacterial photobioreactor, Chemosphere 264 (2021) 128470, https://doi.org/10.1016/J.CHEMOSPHERE.2020.128470.

[24] R. López-Serna, M. Petrović, D. Barceló, Development of a fast instrumental method for the analysis of pharmaceuticals in environmental and wastewaters based on ultra high performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS), Chemosphere 85 (2011) 1390–1399, https://doi.org/10.1016/J.CHEMOSPHERE.2011.07.071.

[25] R. López-Serna, D. García, S. Bolado, J.J. Jiménez, F.Y. Lai, O. Golovko, P. Gago-Ferrero, L. Ahrens, K. Wiberg, R. Muñoz, Photobioreactors based on microalgae-bacteria and purple phototrophic bacteria consortia: a promising technology to reduce the load of veterinary drugs from piggery wastewater, Sci. Total Environ. 692 (2019) 259–266, https://doi.org/10.1016/J.SCITOTENV.2019.07.126.

[26] MAGRAMA, Ministerio de Agricultura, Alimentación y Medio Ambiente, Secretaría General Técnica. Encuesta de Precios de la Tierra, https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/preciosmediosanualesdelastierrasdeusoagrario_2023_tcm30-697862.pdf, 2023.

[27] I. de Godos, Z. Arbib, E. Lara, R. Cano, R. Muñoz, F. Rogalla, Wastewater Treatment in Algal Systems, in: Innovative Wastewater Treatment and Resource Recovery Technologies: Impacts on Energy, Economy and Environment, 2017.

[28] Acién Fernández, F. G., Fernández Sevilla, J. M., Molina Grima, E. Costs analysis of microalgae production, biomass, biofuels, biochemicals: biofuels from algae, Second Edition (2019) 551–566. https://doi.org/10.1016/B978-0-444-64192-2.00021-4.

[29] R. Gutiérrez, F. Passos, I. Ferrer, E. Uggetti, J. García, Harvesting microalgae from wastewater treatment systems with natural flocculants: effect on biomass settling and biogas production, Algal Res. 9 (2015) 204–211, https://doi.org/10.1016/J.ALGAL.2015.03.010.

[30] E. Posadas, A. Muñoz, M.C. García-González, R. Muñoz, P.A. García-Encina, A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters, J. Chem. Technol. Biotechnol. 90 (2015) 1094–1101, https://doi.org/10.1002/jctb.4417.

[31] I.B. Magalhães, A.S.A. de P. Pereira, T.A. Silva, J. Ferreira, M.Q. Braga, E.A. Couto, P.P. Assemany, M.L. Calijuri, Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: a review, J. Water Process Eng. 66 (2024) 105929. doi:https://doi.org/10.1016/J.JWPE.2024.105929.

[32] A.C. de Lima Barizão, L.E. de Oliveira Gomes, L.L. Brandão, I.C.F. Sampaio, I.V. L. de Moura, R.F. Gonçalves, J.P. de Oliveira, S.T. Cassini, Microalgae as tertiary wastewater treatment: energy production, carbon neutrality, and high-value products, Algal Res. 72 (2023) 103113, https://doi.org/10.1016/J.ALGAL.2023.103113.

[33] A. Torres-Franco, F. Passos, C. Figueredo, C. Mota, R. Muñoz, Current advances in microalgae-based treatment of high-strength wastewaters: challenges and opportunities to enhance wastewater treatment performance, Rev. Environ. Sci. Biotechnol. 20 (2021) 209–235, https://doi.org/10.1007/s11157-020-09556-8.

[34] C. Gómez-Serrano, M.M. Morales-Amaral, F.G. Acién, R. Escudero, J.M. Fernández-Sevilla, E. Molina-Grima, Utilization of secondary-treated wastewater for the production of freshwater microalgae, Appl. Microbiol. Biotechnol. 99 (2015) 6931–6944, https://doi.org/10.1007/s00253-015-6694-y.

[35] Z. Arbib, J. Ruiz, P. álvarez-Díaz, C. Garrido-Pérez, J. Barragan, J.A. Perales, Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater, Ecol. Eng. 52 (2013) 143–153, https://doi.org/10.1016/J.ECOLENG.2012.12.089.

[36] V. Matamoros, Y. Rodríguez, J. Albaigés, A comparative assessment of intensive and extensive wastewater treatment technologies for removing emerging contaminants in small communities, Water Res. 88 (2016) 777–785, https://doi.org/10.1016/J.WATRES.2015.10.058.

[37] K. Sukačová, M. Trtílek, T. Rataj, Phosphorus removal using a microalgal biofilm in a new biofilm photobioreactor for tertiary wastewater treatment, Water Res. 71 (2015) 55–63, https://doi.org/10.1016/J.WATRES.2014.12.049.

[38] S.B. Velásquez-Orta, I. Yáñez-Noguez, I.M. Ramírez, M.T.O. Ledesma, Pilot-scale microalgae cultivation and wastewater treatment using high-rate ponds: a meta-analysis, Environ. Sci. Pollut. Res. Int. 31 (2024) 46994–47021, https://doi.org/10.1007/s11356-024-34000-7.

[39] E. Barbera, M. Turetta, E. Sforza, The effect of the internal nutrient quota accumulation on algal-based wastewater treatment: decoupling HRT and SRT to improve the process, J. Water Process Eng. 49 (2022) 103112, https://doi.org/10.1016/J.JWPE.2022.103112.

[40] BOE-A-2007-21092 Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas., (n.d.). https://www.boe.es/buscar/act.php?id=BOE-A-2007-21092 (accessed June 5, 2025).

[41] G. Ruas, M.L. Serejo, P.L. Paulo, M.Á. Boncz, Evaluation of domestic wastewater treatment using microalgal-bacterial processes: effect of CO2 addition on pathogen removal, J. Appl. Phycol. 30 (2018) 921–929, https://doi.org/10.1007/s10811-017-1280-6.

[42] N.A. Buchanan, P. Young, N.J. Cromar, H.J. Fallowfield, Performance of a high rate algal pond treating septic tank effluent from a community wastewater management scheme in rural South Australia, Algal Res. 35 (2018) 325–332, https://doi.org/10.1016/J.ALGAL.2018.08.036.

[43] P. Chambonniere, J. Bronlund, B. Guieysse, Escherichia coli removal during domestic wastewater treatment in outdoor high rate algae ponds: long-term performance and mechanistic implications, Water Sci. Technol. 82 (2020) 1166–1175, https://doi.org/10.2166/wst.2020.233.

[44] R. Muñoz, B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: a review, Water Res. 40 (2006) 2799–2815, https://doi.org/10.1016/j.watres.2006.06.011.

[45] I. de Godos, V.A. Vargas, S. Blanco, M.C.G. González, R. Soto, P.A. García-Encina, E. Becares, R. Muñoz, A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation, Bioresour. Technol. 101 (2010) 5150–5158, https://doi.org/10.1016/J.BIORTECH.2010.02.010.

[46] H. Sousa, C.A. Sousa, L.C. Simões, M. Simões, Microalgal-based removal of contaminants of emerging concern, J. Hazard. Mater. 423 (2022) 127153, https://doi.org/10.1016/J.JHAZMAT.2021.127153.

[47] J.L. Zhou, L. Yang, K.X. Huang, D.Z. Chen, F. Gao, Mechanisms and application of microalgae on removing emerging contaminants from wastewater: a review,

Bioresour. Technol. 364 (2022) 128049, https://doi.org/10.1016/J.BIORTECH.2022.128049.

[48] E. Villar-Navarro, R.M. Baena-Nogueras, M. Paniw, J.A. Perales, P.A. Lara-Martín, Removal of pharmaceuticals in urban wastewater: high rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes, Water Res. 139 (2018) 19–29, https://doi.org/10.1016/J.WATRES.2018.03.072.

[49] C. Escapa, R.N. Coimbra, S. Paniagua, A.I. García, M. Otero, Comparative assessment of diclofenac removal from water by different microalgae strains, Algal Res. 18 (2016) 127–134, https://doi.org/10.1016/J.ALGAL.2016.06.008.

[50] A. Hom-Diaz, A. Jaén-Gil, I. Bello-Laserna, S. Rodríguez-Mozaz, T. Vicent, D. Barceló, P. Blánquez, Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting, Sci. Total Environ. 592 (2017) 1–11, https://doi.org/10.1016/J.SCITOTENV.2017.02.224.

[51] V. Matamoros, R. Gutiérrez, I. Ferrer, J. García, J.M. Bayona, Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study, J. Hazard. Mater. 288 (2015) 34–42, https://doi.org/10.1016/J.JHAZMAT.2015.02.002.

[52] K.Y. Bell, J. Bandy, B.J. Finnegan, O. Keen, M.S. Mauter, A.M. Parker, L.C. Sima, H. A. Stretz, Emerging pollutants – part II: treatment, Water Environ. Res. 85 (2013) 2022–2071, https://doi.org/10.2175/106143013x13698672323308.

[53] V. Matamoros, J.M. Bayona, Removal of pharmaceutical compounds from wastewater and surface water by natural treatments, Compr. Anal. Chem. 62 (2013) 409–433, https://doi.org/10.1016/B978-0-444-62657-8.00012-4.

[54] M. Anastassiades, S.J. Lehotay, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. https://academic.oup.com/jaoac/article/86/2/412/5656996, 2003.

[55] Roccaro, P., Vagliasindi, F.G.A. Membrane bioreactors for wastewater reclamation: cost analysis, current developments in biotechnology and bioengineering: advanced membrane separation processes for sustainable water and wastewater management - case studies and sustainability Analysis (2020) 311–322. https://doi.org/10.1016/B978-0-12-819854-4.00013-7.

[56] D.P. Zagklis, G. Bampos, Tertiary Wastewater Treatment Technologies: A Review of Technical, Economic, and Life Cycle Aspects, Processes 2022, Vol. 10, Page 2304 10 (2022) 2304. doi:https://doi.org/10.3390/PR10112304.

[57] M. Molinos-Senante, F. Hernández-Sancho, R. Sala-Garrido, Economic feasibility study for wastewater treatment: a cost–benefit analysis, Sci. Total Environ. 408 (2010) 4396–4402, https://doi.org/10.1016/J.SCITOTENV.2010.07.014.

[58] E.M. Pajares, L.G. Valero, I.M.R. Sánchez, Cost of urban wastewater treatment and Ecotaxes: evidence from municipalities in southern Europe, Water, Vol. 11, Page 423 11 (2019) 423. doi:https://doi.org/10.3390/W11030423.

[59] Q. Béchet, M. Plouviez, P. Chambonnière, B. Guieysse, Environmental impacts of full-scale algae cultivation, in: Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products, 2017, pp. 505–525, https://doi.org/10.1016/B978-0-08-101023-5.00021-2.

[60] A. Toledo-Cervantes, J.M. Estrada, R. Lebrero, R. Muñoz, A comparative analysis of biogas upgrading technologies: photosynthetic vs physical/chemical processes, Algal Res. 25 (2017) 237–243, https://doi.org/10.1016/J.ALGAL.2017.05.006.

[61] M. del R. Rodero, E. Posadas, A. Toledo-Cervantes, R. Lebrero, R. Muñoz, Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds, Algal Res. 33 (2018) 284–290, https://doi.org/10.1016/J.ALGAL.2018.06.001.