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Scale-down of high-rate algae ponds systems for urban wastewater reuse

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ABSTRACT

In spite of the important environmental and operative advantages of microalgae-based wastewater treatment systems, they present a low level of implementation. Experimental validations in lab scale should reproduce conditions as close as possible to outdoor conditions. In this sense, two experimental reactors were operated under irradiation and temperature levels corresponding to summer and winter conditions found in temperate climates. The configuration of these reactors resulted in mass transfer rates (gas exchange) in the range of the values reported for industrial scale outdoor facilities. While superficial biomass productivity was in the range of previously reported experiences (between 5.7 and 22 g m^{-2} d^{-1}, in winter and summer conditions, respectively), pollution removal efficiencies reached the values required for wastewater discharge: >78 % of CODt, >96 % of NH_4^+ and >79 % of PO_4^{3-}. Unlike previous experiences, hydraulic retention time (HRT) was maintained constant (3 days) in both seasonal conditions, reaching sufficient pollution removal and biomass productivity. The pathogen concentration reached in the final effluent was compatible with the reclaimed water standards in spite of the absence of ultraviolet light in the illumination system, evidencing the relevance of the environmental conditions created by microalgae metabolism in the disinfection process. The characterization of the microbial population revealed the presence of bacteria responsible of organic matter removal, ammonia oxidation and microalgae commonly found in wastewater systems.

1. Introduction

Microalgae cultivation can provide a low-cost treatment of domestic wastewater. The photosynthetic capacity of these microorganisms releases the oxygen needed for organic matter oxidation, while nutrients are assimilated into algae biomass, resulting in very low concentrations of these pollutants in the treated effluent [1]. In addition, pathogenic bacteria concentration drastically decreases during the algae cultivation as a result of the particular conditions imposed by phototrophic metabolism and photobioreactor conditions [2]. Consequently, high quality effluents with low levels of chemical and biological pollution are produced with very low operational costs in algal-based systems. In spite of the environmental and economic advantages, few full-scale microalgae based systems are currently in operation. Since the first experiences reported in early 1960s, only a handful of large scale facilities, based on raceway reactor configuration, have been reported for domestic wastewater treatment [3]. This technology is regarded by the water industry as an untested alternative with a strong environmental dependence, since the oxygenation rates are driven by light availability and temperature, which in turn, depend on plant location and seasonality. In spite of their low level of implementation, algae-based treatment systems are currently investigated based on their potential in the context of circular economy, which prioritize systems able to provide carbon and nutrient recovery and production of valuable biomass [4].

In this context, research studies can be differentiated in two main strategies: outdoors demonstration assays conducted under long-term operation and lab-scale systems devoted to study specific issues of the bioprocess (e.g. rates of pollutant removal, biomass characteristics, etc.). A wide range of conditions have been tested in lab-scale systems depending on the experimental set-up used: light source, gas-liquid mass

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transfer, mixing system and photobioreactor geometry, which ultimately impact on algal growth and pollutant recovery [5,6]. In this scenario, a considerable amount of published experiences at bench scale do not reproduce outdoor conditions. Reactors are illuminated at constant irradiation values, temperature is kept at continuous levels and mass transfer between the atmosphere and bulk liquid is normally not considered or determined. Therefore, the results reported by different lab-scale experiments cannot be easily compared or extrapolated to pilot outdoor experiences. In this context, the general performance, in terms of pollution removal or microalgae biomass production, can easily under or overestimate the capacities prevailing in demonstration studies [7,8]. Therefore, experimental set-ups must be carefully designed in order to better mimic the conditions found in outdoor photobioreactors devoted to wastewater treatment. In this regard, high rate algae ponds (HRAPs), shallow mixed lagoons arranged in meandering configuration, are the only algae production system able to treat wastewater at a competitive operational cost [4,9]. Therefore, lab-scale photobioreactors should try to reproduce the conditions prevailing in outdoors HRAPs in order to provide realistic data in terms of resource recovery. Unfortunately, most lab-scale algal systems are operated at constant temperature and radiation, which will never provide an accurate description of systems performance under outdoors conditions.

In this work, domestic wastewater treatment was conducted in lab-scale photobioreactors under conditions prevailing in outdoor HRAP reactors. For this purpose, an experimental set up that reproduced the seasonal and daily variations of light intensity and temperature were investigated in an open reactor. In the same way, the mass transfer characteristics of conventional full scale HRAP were mimicked in the reactors. Wastewater treatment was evaluated in terms of nutrient, organic matter and bacterial pathogens removal, with the ultimate goal of water reuse after treatment. The microbial population structure of the algae-bacteria consortia was analyzed under the different conditions tested in order to increase the understanding HRAPs devoted to domestic wastewater treatment.

2. Materials and methods

2.1. Experimental set up

The experimental set up was located indoors at the laboratory of Energy and Physics Department of Valladolid University in Campus of Soria. The system treated real pretreated wastewater from the municipal WWTP of Garray (Soria, Spain). This facility was equipped with a pretreatment consisting of large solid removal by screening. The laboratory system was composed of two 2.15 L cylindrical PVC photobioreactors (diameter = 12.5 cm; height = 28 cm) operated at constant depth of 20 cm and two conical settlers of 0.7 L (diameter = 8 cm; height = 23 cm) connected to each photobioreactor (Fig. 1). The surface of the photobioreactors were illuminated with a day simulator program consisting of two LED module PHILIPS 94 V covering a total surface of 0.16 m^2 (length = 40 cm; width = 23 cm; 140 LEDS for module). LED modules were controlled by means of a programmable electronic driver (Philips LED Xitanium 8173345). Daily and seasonal light intensity variations corresponding to two conditions: winter and summer were programmed with an Arduino device (IDE) that modulated the light intensity by means of a Pulse Width Modulation (PWM). In this way, current delivered by the drivers was controlled in order to generate the daily and seasonal light intensity variations corresponding to winter and summer according to the model of light horizontal irradiations (see next section). Light intensity was calibrated by means of a pyranometer LI-250A (LI-COR, EE. UU). Reactor's temperature was controlled by a thermostatic bath mimicking the natural fluctuations with a peak in central hours of the day. 100 mL of microalgae culture obtained from a previous experiment were introduced at the beginning of the experiment in each reactor. The photobioreactors were fed with two pumps (WATSON MARLOW 313S) at an inlet flowrate of 0.7 L d^{-1} from a 5 L homogenization tank resulting in hydraulic retention times of 3 days. Wastewater was fed during 6 periods of 5 min during the light period, with a gap to 115 min between them, and 2 periods of 5 min during the night with a gap to 175 min. In this manner, most of water entered in the treatment systems during the light period, mimicking the conditions of wastewater treatment facilities Evaporation rates were determined by measurement of the decrease in depth during batch mode experiments at

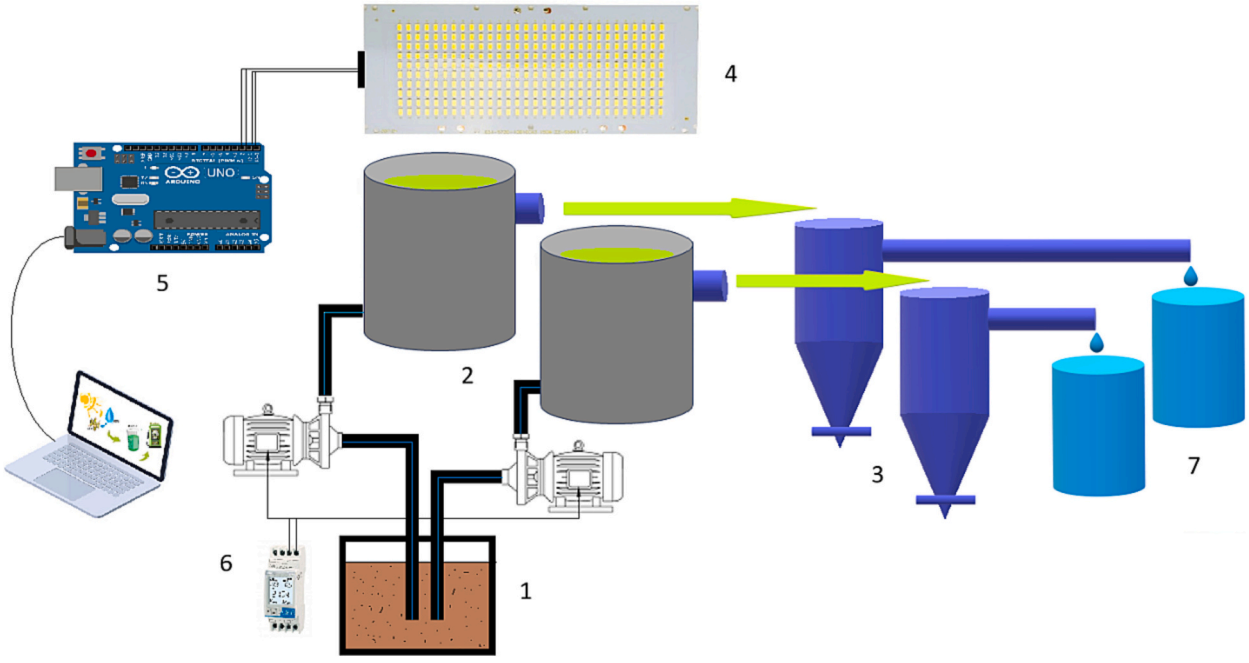

Fig. 1. Schematic diagram of the experimental setup. (1) Wastewater INLET (2) Photobioreactors for wastewater treatment, (3) Conical settlers, (4) LED module, (5) Illumination program System of Arduino (6) Pumps programed system, (7) OUTLET.

the tested environmental conditions. The rates of evaporation were used in the mass balances and biomass productivity calculations [3]. The photobioreactors were operated for 180 days. Liquid samples were collected every two days to monitor photobioreactor performance.

2.2. Mass transfer determination in photobioreactors

The rates of oxygen gas-liquid exchange between the bulk liquid and the atmosphere were determined following a standard procedure of volumetric mass transfer coefficient. The volumetric mass transfer coefficient, K_La_L, was measured using the dynamic gassing-out method [10]. Dissolve oxygen was removed by reaction with sodium sulphite and system was left under mixing conditions. The dissolved oxygen concentration (C) versus time (t) was recorded until close to saturation (Fig. 2). The following equations were used in the procedure described to determine K_La_L (Eqs. (1) and (2)).

$$\frac{dC_L}{dt} = K_L a_L (C^* - C_L) \tag{1}$$

$$ln\left(\frac{C^* - C_L}{C^* - C_0}\right) = -K_L a_L \cdot t \tag{2}$$

where C_L and C^* stand for the oxygen concentration in the bulk aqueous phase and the equilibrium concentration with the oxygen in the atmosphere, respectively, and C_0 represents the initial dissolved oxygen concentration.

2.3. Operational conditions: model for horizontal light irradiation

Two operational conditions were implemented to evaluate domestic wastewater treatment efficiency, summer and winter. The solar radiation was estimated as a function of its location and position following the models described by [11]. Azimut (α) and altitude angles (γ) were calculated as function the latitude (ϕ), hour angle (ω) and declination (δ) using the following equations (Eqs. (3)–(5)):

$$\alpha = sin^{-1}(sin(\delta)sin(\phi) + cos(\delta)cos(\omega)) \tag{3}$$

$$\gamma = cos^{-1}\left(\frac{sin(\delta)cos(\phi) - cos(\delta)cos(\omega)sin(\phi)}{cos(\alpha)}\right) \tag{4}$$

$$if\ sin(\omega) > 0\ then\ \gamma = 360 - \gamma\ \ if\ sin(\omega) \le 0\ then\ \gamma = \gamma \tag{5}$$

The hour angle (ω) was calculated as function the solar time (h_s) (Eq. (6)) and the sun declination solar time (δ) was obtained corresponding the day of the year (n) according (Eq. (7)).

$$\omega = 15(12 - h_s) \tag{6}$$

$$\delta = 23.45sin\left(\frac{360(284 + n)}{365}\right) \tag{7}$$

In order to validate the results with outdoor experiments the data corresponding with Soria environmental conditions were used in this simulation: altitude of 1063 m and latitude angle 41°. The hourly global radiation on a horizontal surface, G_s, can be calculated as a function global radiation (G_0), the day of the year (n), the latitude (ϕ), hour angle (ω) and the sun declination solar time (δ) (Eqs. (7) and (8)).

$$G_s = G_0\left(1 + 0.033cos\frac{360n}{365}\right)(cos\,\phi\,cos\,\delta\,cos\,\omega + sin\,\phi\,sin\,\delta) \tag{8}$$

The calculation of daily solar radiation was done by integrating Eq. (8) over the period from sunrise to sunset. Daily light intensity variations were transformed into two polynomic equations, corresponding to summer and winter conditions, used to modulate the current delivered to the LEDs module (Fig. 2). Calibration and verification of the light intensity was performed by means of a pyranometer LI-250-LICOR.

The global radiation and the daily extraterrestrial radiation on a horizontal surface and the photobioreactors temperature are given by Fig. 2:

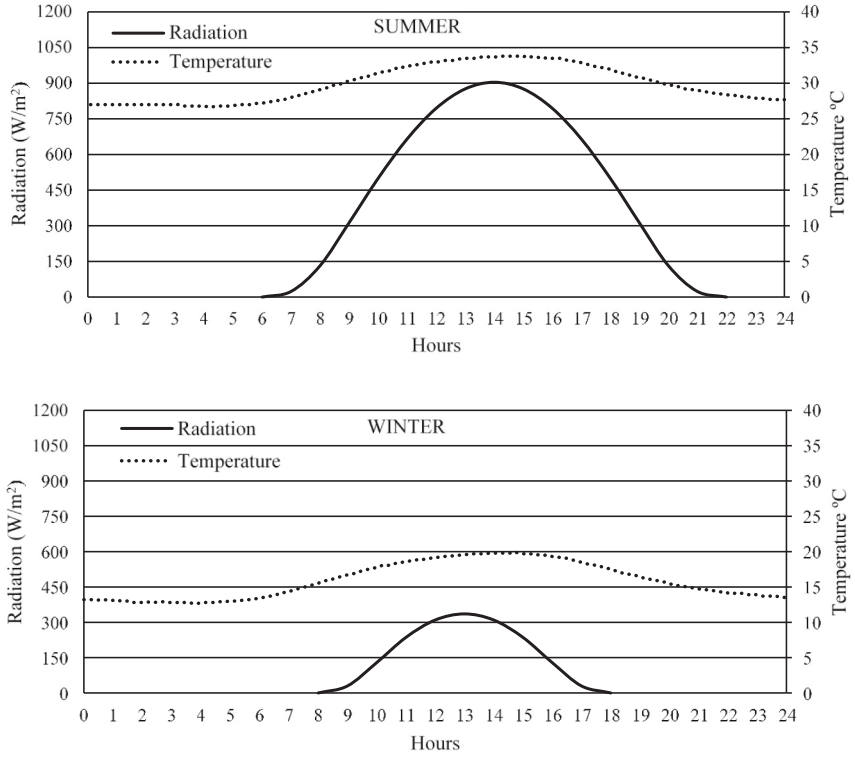

Fig. 2. Experimental data of horizontal radiation on the surface of the photobioreactors (—) and temperature of the bulk liquid (···) during a cycle.

2.4. Analytical methods

Wastewater treatment efficiency was evaluated using COD, NH_4^+, PO_4^{3-}, total and volatile suspended solids (TSS and VSS) as proxy parameters according to Standard Methods [12]. The concentrations of NO_2^-, NO_3^-, IC and anions through isocratic mode with according to Standard Methods, total carbon (TC) and total nitrogen (TN) [12]. Additionally, optical density at 550 nm and 680 nm were also used for estimation of biomass concentration in a Genesys 10s UV–vis spectrophotometer (see Supplementary Material). All the analyses were done in duplicate and results are given as average values with their associated standard deviation. Biomass productivity was determined as total suspended solids (TSS) considering the outlet flow rate and the photobioreactor surface. Organic matter removal was estimated using total and soluble Chemical Oxygen Demand (COD) concentration. Soluble COD was measured after filtration through 0.45 μm of nylon filter. Dissolved oxygen (DO), pH and temperature were daily monitored under summer and winter conditions, which allowed continuous data acquisition using a Consort C3010 multi – parameter analyzer.

E. coli and coliform concentration in influent and effluent of the photobioreactors were determined using the protocol described in the ISO 9308-1:2014 [13] with Chromocult® Coliform Agar (CCA). The method is based on membrane filtration for 100 mL and Petri dishes cultivation, subsequent culture on a chromogenic coliform agar medium, and calculation of the number of target organisms in the sample. The method was used for detection and enumeration of *Escherichia coli* and coliform bacteria. Values are indicated in the text, table and figures with standard deviation.

2.5. Microbial population structure determination

Samples for characterization of the microbial population were taken under steady state in each experiment. The description of the communities is included in the Section 3.5. A total of 50 ng of DNA was amplified following the 16S Metagenomic Sequencing Library Illumina 15044223 B protocol (ILLUMINA). Three different regions were amplified, using three sets of primers. In order to study bacterial communities, region V3-V4 of the 16S rRNA gene was amplified using 341F-805R primers [14]. Region V4 of 18S was amplified to study microalgae communities, using TAReuk454FWD1 forward primer and TAReukREV3 reverse primer. Libraries were pooled before sequencing on the MiSeq platform (Illumina), 300 cycles paired reads configuration. Sequencing and bioinformatics analysis were performed by Life sequencing S.L. (University of Valencia, Spain).

3. Results and discussion

3.1. Scale-down of high rate algae pond

The experimental set up reproduced environmental conditions typically found in outdoor HRAPs during clear sky days. Maximum temperatures at midday reached values of 33.9 °C and 13.8 °C in summer and winter, respectively. Dark period showed temperatures of 26 and 13 °C for the same conditions. In this experiment, the values empirically determined for the gas-liquid volumetric oxygen mass transfer coefficient (0.39 h^{-1}) were in the same range that those reported for outdoor HRAPs used for wastewater treatment. For instance, reported values of 0.41 h^{-1} in an outdoor 32 m^2 raceway pond. However, significantly higher values were reported by [15], ranging from 0.68 to 0.76 h^{-1} in a 1000 m^2 HRAP, while [16] measured a K_La_L coefficient of 1.8 h^{-1} in a semi-industrial scale pond of 100 m^2. However, these divergences are probably due to the strong impact of the reactor configuration and paddle wheel engine in the gas-liquid mass transfer through the pond surface. In this case, used a multiple bended pond and [16] HRAP was equipped with sumps for reaeration. In this contest, the values herein reported are close to the simplest HRAP configuration.

3.2. Biomass productivity

Biomass concentration averaged in 0.95 ± 0.10 g L^{-1} TSS and 0.44 ± 0.03 g L^{-1} TSS g L^{-1}, in summer and winter conditions respectively. Areal biomass productivities reached an average value of 22.0 ± 2.6 g VSS·m^{-2}·d^{-1} under summer conditions, while in winter conditions a considerably lower productivity of 5.7 ± 2.6 g VSS·m^{-2}·d^{-1} was detected. Similar values of superficial productivity were obtained by [3] in 9.6 m^2 raceway ponds located in Chiclana de la Frontera (South Spain), treating domestic wastewater at 3 days of HRT (23.9 ± 4.1 and 7.5 ± 1.9 g·m^{-2}·d^{-1}, in summer and winter, respectively). However, considerably lower values have been reported by other authors. An average superficial productivity of 12.7 g·m^{-2}·d^{-1} in a 31.8 m^2 pond operated at a HRT ranging from 4 to 8 days were measured in a pond located in Hamilton (New Zealand) during summer [14]. At this point it must be stressed that the recreation system herein presented was operated under clear sky conditions without the decrease in irradiation due to meteorological events (such rain or clouds). In the case of winter conditions, similar biomass productivities have been reported by [14]: 5.1 g·m^{-2}·d^{-1} at HRTs of 6 days. Modification of the HRT along the year is a widely spread strategy applied to avoid biomass wash-out during the harsh conditions of winter, when the growth rate of microalgae exhibit lower values. However, 3 days of HRT was sufficient to maintain algae production and pollutant removal in the work herein presented. These findings have a particular significance since wastewater treatment facilities are preferably operated at constant flow. In this sense, the increase of the wastewater retention time during winter could be a convenient strategy for experimental systems but would not comply with requirements of fully operative wastewater treatment facilities.

3.3. Wastewater treatment performance

The photobioreactors herein operated supported a very high efficiency in the removal of COD, TSS, N-NH_4^+ and P-PO_4^{-3} in spite of the different conditions applied. Table 1 shows the results obtained for two photobioreactors analysis of the influents and effluents of different seasonality.

Organic matter was efficiently removed in the HRAPs with efficiencies higher than 80 % for soluble and total COD. Photosynthetically based oxidation processes mediated by heterotrophic microalgae and bacteria were responsible of this removal, since the aeration rates due to oxygen exchange from the atmosphere were clearly insufficient to cope with the chemical oxygen demand load. Total COD concentration in the effluent was slightly higher in summer conditions as consequence of the presence of suspended algae in the final effluent and the higher evaporation rates from the pond surface (Fig. 3). This effect was also measured in terms of TSS in the final effluent with values of 16 and 35 mg/L in winter and summer, respectively. This fact was directly related to the higher biomass average concentration reached under summer conditions in the reactors (980 mg/L vs. 440 mg/L, respectively). The photobioreactors supported removal efficiencies above 80 % in all the parameters monitored under winter and summer, except for CODt and P-PO_4^{-3} in summer conditions, which presented slightly lower values. In this sense, the experimental plant complied with the discharge limits for urban wastewater according to the European Directive 91/271/EEC. This regulation requires a COD removal efficiency > 75 % or COD concentrations < 125 mg·L^{-1}, a N-NH^{+4} removal efficiency > 80 % or <15 mg N·L^{-1}, a P-PO_4^{-3} removal efficiency of 70–80 % or <2 mg P·L^{-1} and a TSS removal efficiency >90 % or <35 mg TSS·L^{-1} in the effluent. Similar values have been reported in similar experimental systems. For instance [17] achieved a COD removal efficiency of 94 % in a 250 m^3 pond treating real wastewater under summer/winter conditions at a HRP of 365 days.

Ammoniacal nitrogen was efficiently removed under both environmental conditions (>90 %), resulting in very low concentrations in the final effluent (Fig. 4). However, winter conditions were characterized by

Table 1
Comparison analysis of the influents and effluents of different seasonality in the photobioreactors.

Parameter (units)	Sample point				Removal efficiency %	
	Winter		Summer		Winter	Summer
	IN	OUT	IN	OUT		
CODt (mg/L)	181.72 ± 92.83	31.13 ± 11.84	387.37 ± 85.28	80.06 ± 31.71	81.59 ± 9.32	78.08 ± 8.61
CODs (mg/L)	113.88 ± 81.99	5.22 ± 2.87	177.21 ± 23.62	18.66 ± 7.36	82.837 ± 10.73	89.18 ± 4.60
N-NH^{+4} (mg/L)	24.59 ± 10.61	0.68 ± 0.36	16.47 ± 7.05	0.47 ± 0.13	97.00 ± 2.01	96.64 ± 1.60
N-NO_2^- (mg/L)	2.20 ± 1.15	0.71 ± 1.85	0.00 ± 0.00	1.71 ± 2.22		
N-NO_3^- (mg/L)	2.01 ± 1.52	10.03 ± 8.78	0.00 ± 0.00	0.00 ± 0.00		
P-PO_4^{-3} (mg/L)	9.38 ± 5.22	0.90 ± 0.07	7.19 ± 6.87	0.86 ± 0.06	83.20 ± 10.91	79.19 ± 6.87
TSS (mg/L)	157.78 ± 7.03	16.47 ± 7.04	248.5 ± 88.80	35.34 ± 13.28	94.26 ± 3.49	83.72 ± 8.54
E. coli (CFU/100 mL)	1.98E+4 ± 1.59E+3	212.50 ± 17.68	6.28E+5 ± 1.30E+5	24.93 ± 1.62	98.93 ± 4.98E−1	100.00 ± 3.51E−3
Enterobacter A. (CFU/100 mL)	1.09E+4 ± 1.34E+4	232.50 ± 84.11	5.07E+5 ± 1.49E+5	28.00 ± 9.02	99.79 ± 4.35E−2	99.98 ± 4.80E−3
Enterococcus F. (CFU/100 mL)	8.31E+3 ± 3.80E+3	290.00 ± 14.14	2.32E+5 ± 5.66E+4	9.42 ± 0.31	94.84 ± 2.51E−1	99.95 ± 2.36E−2

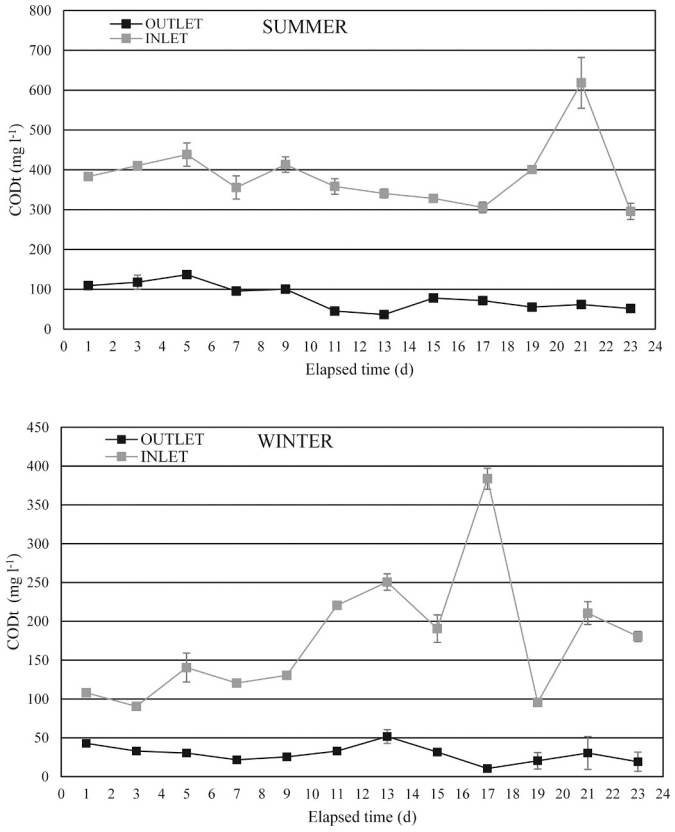

Fig. 3. Evolution of concentration of total Chemical Oxygen Demand (tCOD) in inlet (■) and outlet (■) samples of the photobioreactors.

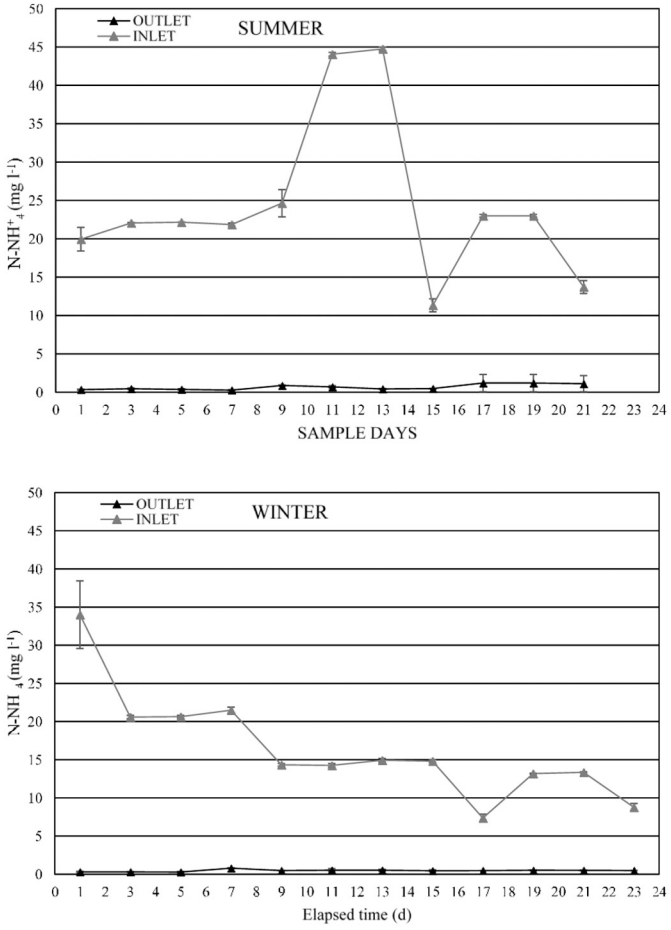

Fig. 4. Evolution of N-NH_4^+ concentration in inlet (■) and outlet (■) samples of the photobioreactors.

a stable nitrification process stablished from sample day 8 of operation with average concentrations of 18 mg/L. On the other hand, the nitrate and nitrite concentrations during summer conditions exhibited very low values at the beginning of the experiment and a decreasing trend along the experiment, resulting in negligible concentrations from sample day 9 onwards (Fig. 5). The order of priority for the consumption of nitrogen sources for microalgae was, as expected, ammonium (NH_4^+) and then (NO_2^-) and (NO_3^-) [18]. Therefore, once ammonia nitrification was stablished, the oxidized forms of nitrogen remained in the final effluent since ammonium was uptaken in first place. The occurrence of nitrification in HRAPs has been previously documented, but conditions conductive for nitrifiers are not well understood. In the case of domestic wastewater treatment processes requiring high nitrogen removal efficiencies, this bioprocess should be avoided since the presence of NO_2^- and NO_3^- results in high concentrations of nitrogen and therefore the discharge limits cannot achieve. Alternatively, it can be coupled to a previous anoxic tank using an internal liquid recirculation, as engineered by García and co-workers [19]. The development of nitrifying bacteria depends on the availability of substrates consumed by microalgae (CO_2, HCO_3^-, NH_4^+ and O_2). In this sense, winter conditions reduce algae growth rate and consequently the uptake of inorganic carbon and NH_4^+, increasing the availability of these substrates for nitrifiers [18]. Finally, high pH values registered along the experiment probably caused ammonia volatilization (see supplementary material).

3.4. Pathogen removal

Algae-based systems can provide high pathogenic bacteria removals

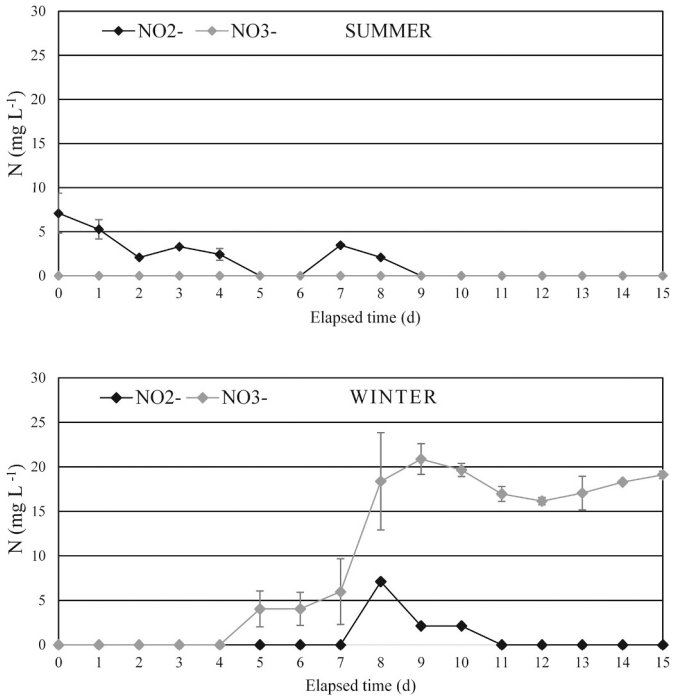

Fig. 5. Evolution of N-NO_3^- (▒) and N-NO_2^- (■) concentration in and outlet samples of the photobioreactors.

as consequence of the environment created by microalgae and the exposure to solar irradiation. Thus, secondary and tertiary treatment take place simultaneously and the final effluents can be reused. High levels of disinfection were achieved in both conditions. Indeed, summer conditions resulted in an effluent with an *E. coli* concentration of 24.93 ± 1.62 CFU·100 mL^{-1}, while winter conditions decreased the level of disinfection with average value of 212.50 ± 17.68 CFU·100 mL^{-1}. Under summer conditions, the level of disinfection was compatible with use of water for irrigation of crops in which the edible part is produced above ground and is not in direct contact with water (class B in the reclaimed water classification) according to the European Regulation (2020/741). Winter conditions were characterized by lower levels of disinfection. However, the concentrations of *E. coli* reached the limits stablished for drip irrigation, classified as class C in the regulation. Similarly, removal of the other pathogen bacteria (not included in the normative) reached considerable high values in winter and summer conditions: >99.9 % of *Enterococcus A.* in winter and summer conditions, and between 94 and 99 % of *Enterococcus F.*, for the same periods. The main mechanisms of pathogen decay during algae culture in HRAPs are exposure to light, and cellular damage caused by high pH and oxygen oversaturation and by the presence of toxic algal metabolites [20]. Some authors have suggested that UV light exposure is the main mechanism of pathogen removal since most of bacteria are sensible to DNA damage mediated by UV light [21]. However, the light source used in this experiment only provided light in the visible spectrum. Therefore, pathogen decay during these experiments was probably due to a combination of high pH (8.98), DO and potential inhibitory compounds excreted by microalgae. In this context, [22] suggested that pathogen decay in algae systems could be caused by the formation of reactive oxygen species (ROS) mediated by light (visible and UV spectrum) exposure. Other authors have suggested that dissolved organic molecules present in wastewater act as photosensitizers that are excited by photons of different wavelengths, creating ROS that provides disinfection. Other mechanism of pathogen removal documented include predation by viruses and protozoa, and the release of algae toxic metabolites [20]. In any case, the result herein presented showed that high levels of disinfection can be achieved without UV irradiation. These findings are of relevance since it has been demonstrated that high levels of pathogen bacteria removal can be reached even at very low UV irradiations, conditions that can be found during winter or at high latitudes.

3.5. Microbial communities

The analysis of microalgae and bacteria populations revealed the impact of climate conditions over the structure and diversity of the microbiome (Fig. 6). In the case of algae, winter conditions were characterized by the dominance of *Desmodesmus opoliensis* and *Scenedesmus vacoulatus*, which accounting for 55.8 and 35.0 % of total microalgae, respectively. While summer conditions resulted in a higher diversity, *Desmodemus opoliensis* reduced its abundance to 27.6 % and *Scenedemus vacoulatus* to 25.4 %. Other *Desmodemus* species presented an abundance of 29.1 %. *Tetradesmus deserticola* and *Neochloris conjunta* showed relative abundances of 7.2 and 8.8 %, respectively. The increase in diversity during summer conditions has been also reported under outdoor experiments [23]. The algae genera found in this experiment have been previously reported in domestic wastewater treatment experiments and are characterized by a high tolerance towards organic pollution concentration [24].

Bacteria population showed a high diversity in both summer and winter conditions. Aerobic heterotrophs were found in both conditions with genera *Gemmatacea*, *Planctomycetales*, *Pirellulacea* and *Cryobacterium* (Fig. 6). An endosymbiotic bacterium of the protozoa *Acanthamoeba* was found in either sample, evidencing the presence and abundance of the protozoa. Some of the organisms found in higher proportion during winter have been related with psycrophylic conditions (*Pirellulacea* and *Cryiobacterium*). An Ammonia Oxydizing Bacteria (AOB) belonging to the genus *Chitinophagea* was found only in samples withdrawn in winter and accounted with 7.01 % of total bacteria. This fact can be directly associated with the high levels of nitrification found in winter conditions. As described earlier, light limitation reduce competence between microalgae and nitrifiers for key substrates such as ammonia and inorganic carbon and promoted the development of this autotrophic bacterial group.

4. Conclusions

The environmental conditions encountered in outdoor HRAPs were reproduced in bench scale mimicking winter and summer irradiations and temperatures in temperate climates. The experimental system achieved areal biomass productivities similar to those reported under outdoor conditions. Constant HRT of 3 days were applied without biomass washout even under winter conditions. Overall, pollutant removal from real wastewater was sufficient to accomplish the discharge regulation in terms of organic matter and nutrient removal. Unexpectedly, the high levels of nitrification during winter reduced the total nitrogen removal. High levels of pathogen removal were achieved, compatible with the reclaimed water normative, despite no UV light was provided, which suggested the occurrence of other disinfection mechanisms. The analysis of the microbiome revealed a higher diversity of microalgae in summer and the dominance of aerobic heterotrophs among the bacterial population. Nitrification levels were correlated with the presence of one ammonia oxidizing bacterial species.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ignacio de Godos Crespo reports financial support was provided by European Commission. Ignacio de Godos Crespo reports was provided by Government of Castile and León.

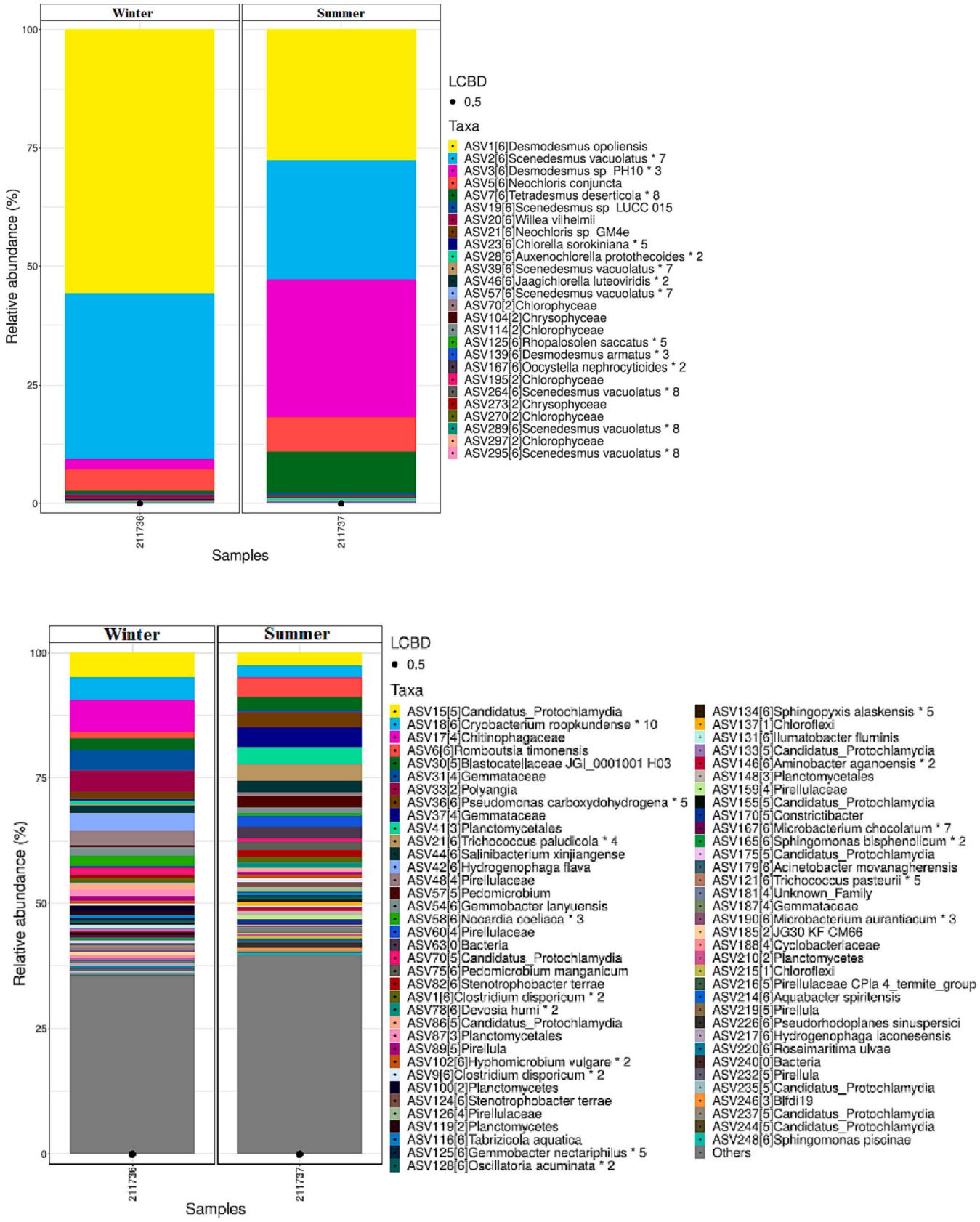

Fig. 6. Analysis of microalgae and bacteria populations.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2023.104342.

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