Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways

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ABSTRACT

The influence of pH (7, 8 and 9) and CO_2 source (pure CO_2 or CO_2 from flue gas) on both the performance of secondary domestic wastewater treatment and biomass productivity and composition in three outdoors pilot raceways was evaluated for 6 months. Average COD, TN, TP and *E. coli* removal efficiencies of 84±7%, 79±14%, 57±12% and 93±7%, respectively, were recorded. The influence of pH on wastewater treatment was negligible, while the supply of CO_2 from flue gas supported higher COD, TOC and TP removals. Biomass productivities ranged from 4±0 g m^{-2} d^{-1} in December to 17±1 g m^{-2} d^{-1} in July. The highest C, N and P biomass contents (64.8%, 12.6 % and 2.4 %, respectively) were recorded when flue gas was supplied. Finally, while the protein content in the biomass remained constant (38.2±3.3%), the lipid and carbohydrate contents ranged from 5.8% to 23.0% and from 38.0% to 61.2%, respectively.

Keywords: algal-bacterial symbiosis; biological wastewater treatment; flue gas; microalgae biomass composition; microalgae biomass production.

1. Introduction

Wastewater management represents an increasing concern worldwide as a result of the exponential human population increase and the rapid industrialization since the mid-20th century. The uncontrolled disposal of domestic and industrial wastewaters into the environment causes severe pollution problems such as eutrophication or oxygen depletion in lakes and rivers, which makes wastewater treatment mandatory [1]. Unfortunately, conventional wastewater treatment technologies present some techno-economic limitations [2]. For instance, process aeration represents 45-75% of the total operation costs in an activated sludge wastewater treatment plant (WWTP) [3], while anaerobic digestion entails a poor nutrient removal [4]. In this context, microalgal-bacterial processes constitute a sustainable and cost-effective alternative to conventional technologies due to their free oxygenation potential and efficient nutrient removal [5]. This green biotechnology is characterized by the oxidation of the organic pollutants present in the wastewater to CO_2 by heterotrophs and by the assimilation of nutrients as a valuable algal-bacterial biomass, which can be further used as a biofertilizer and/or as a feedstock for biofuel production [6,7]. As a result of CO_2 fixation in the presence of light, microalgae photosynthetically provide the O_2 needed by heterotrophs and nitrifiers for the oxidation of organic pollutants and NH_4^+ [8].

Microalgae-based processes were first implemented in the mid 1950s in California for domestic wastewater treatment in algal ponds called raceways (RWs) [9]. RWs are currently the most economic photobioreactor configuration for microalgae cultivation, despite their lower algal biomass productivities when compared to closed photobioreactors [10]. RWs consist of shallow ponds (0.1-0.4 m deep) divided into two or four water channels in order to allow liquid mixing and circulation, which is often provided by paddlewheel mechanical agitation [11]. Since their early applications, RWs have supported a cost-effective organic matter and nutrient removal from domestic,

industrial and livestock wastewaters [2, 12, 13]. However, the low C/N/P ratio in most wastewaters, compared to the algal-bacterial biomass composition ratio (≈100/18/2), often limits the efficiency of nutrient removal in microalgae-based wastewater treatment processes due to a carbon deficiency [9, 14, 15]. In this regard, an external CO_2 addition into the mixed liquor could enhance algal-bacterial biomass productivities and consequently the recovery of nutrients from wastewaters [16]. CO_2 addition would also prevent the rise in pH in the mixed liquor of the RWs mediated by photosynthetic activity, and therefore mitigate nitrogen losses by $N\text{-}NH_3$ stripping and phosphorus precipitation [1, 17]. However, despite the potential of this synergistic process integration, the number of outdoors studies assessing at semi-industrial scale the performance of wastewater treatment supported by CO_2 addition is scarce, with the few studies available mainly focused on tertiary wastewater treatment [14, 16].

The present work assessed the performance of three outdoors semi-industrial RWs operated in parallel during secondary domestic wastewater treatment for 6 months (July-December) at three different pHs (7,8 and 9) controlled by the addition of pure CO_2 and CO_2 from real flue gas. The operation of the RWs was also monitored without pH control in order to evaluate the reproducibility of process performance and to serve as control.

2. Materials and methods

2.1 Microorganisms

The RWs were inoculated with *Scenedesmus* sp. previously cultivated in an outdoors thin layer RW and with activated sludge from the WWTP of El Ejido (Almería, Spain) at total suspended solid (TSS) concentrations of 2,500 and 4,500 mg L^{-1}, respectively. Under the particular environmental conditions of Almería, *Scenedesmus* has been consistently shown as the dominant microalga species, which supports the selection of

this microalga for the inoculation of our raceways [18]. In addition, *Scenedesmus* has been also consistently reported as a microalga species commonly found in photobioreactors treating domestic wastewater [19] (Photograph 2a, supplementary material).

2.2 Experimental set-up

Experiments were conducted in three outdoor raceways (RW1, RW2 and RW3) located at Estación Experimental Las Palmerillas, property of Fundación CAJAMAR (Almería, Spain) (Fig. 1a; Photograph 2b and 2c in Supplementary Data). RW1, RW2 and RW3 consisted of three polypropylene algal ponds of two 6-m length channels, 0.6-m width connected by 180º bends at each end, with 8.33 m^2 of illuminated surface and 10 cm of depth. Guide vanes made of polypropylene were placed in the bends of the photobioreactors. The total working volume in RW1, RW2 and RW3 was 700, 800 and 850 L, respectively. The main difference in the photobioreactor volume was the setting of a 1-m depth sump to improve CO_2 mass transfer in RW2 and RW3 (Fig. 1b) [20]. The sump volumes in RW2 and RW3 were respectively 100 and 150 L. Culture mixing in the RWs was provided by a six bladed paddlewheel driven by an electric motor (Motovario, Italy), which supported a liquid recirculation velocity of 20 cm s^{-1}.

<Figure 1>

2.3 Operational conditions

RW1, RW2 and RW3 were initially filled with tap water, and inoculated with *Scenedesmus* sp. and activated sludge at 30% and 10% of their total working volume, respectively. RW1, RW2 and RW3 were initially fed in semi-continuous mode during the first month of operation between 9 and 12 a.m. with primary domestic wastewater using a S-561 82 Hysqvarna AB pump (Sweden) at a hydraulic retention time (HRT) of

3.3±0.2 d, and maintained at a pH of 8 (automatically controlled via pure CO_2 sparging) in order to acclimate the microorganisms to cultivation in wastewater. Automatically controlled valves (Cepex, L10, Spain) for wastewater flow rate control were installed after this acclimation period (≈ 34 days) in order to feed the wastewater into the RWs for 12 hours a day. Four operational stages were tested, whose main operational characteristics and objectives are showed in Table 1. Pure CO_2 (stage I) or CO_2 from flue gas (stages II and III) were supplied at the bottom of the sumps (or at the bottom of RW1) through a 25 mm diameter polyethylene diffuser to control the pH. The pH control set points in RW1, RW2 and RW3 were 9, 8 and 7 during stages I and II (operated at a HRT of 2.7±0.1 and 2.8±0.2 d, respectively), while pH 8 was the set point in the three RWs during stage III (operated at a HRT of 6.7±0.4 d) (Table 1). The pH set point of 9 was established in RW1 due to the lower CO_2 mass transfer efficiencies previously recorded in RWs without sump [11]. CO_2 and flue gas supply was regulated by a solenoid on/off valve automatically opened when pH increased over the set point. Air was continuously sparged into the systems in the absence of CO_2 supply to avoid O_2 accumulation into the raceways, and therefore microalgae inhibition due to photo-oxidation [21]. The CO_2, flue gas and air flow rate sparged into the reactors was maintained at 20 L min^{-1} via a mass flow controllers (PF 725S-F01-F, SMC, Tokyo, Japan). Stage IV involved process operation at a HRT of 6.0±0.3 d in the absence of pH control. Operational conditions in each stage were maintained until a steady state was reached (constant values of TSS, maximum quantum yield (F_v/F_m) and culture absorbances at 680 nm).

<Table 1>

The parameters monitored on line and logged every 6 minutes in the RWs were the pH, temperature and dissolved oxygen (DO) concentration in the mixed liquor, the

composition of CO_2 in the exhaust flue gas, the duration of CO_2 valve opening (FG line, Italy) and the impinging irradiation at the RWs surface. pH was measured by pH probes (Crison, Spain), temperature and DO in the medium were determined with In Pro 6050/120 oxygen sensors (Mettler Toledo; Spain) and the impinging irradiation with a pyranometer G-54 (LI-COR, USA). The composition of CO_2 in the sparged flue gas at the surface of the raceways was measured using GMM 220 carbon dioxide sensors (Vaisala, Finland) coupled to a fume hood. This measurement took place only when using flue gas since the outlet gas when pure CO_2 was sparged for pH control contained CO_2 concentrations above the maximum concentration measured by GMM 220 (20%). The wastewater influent flow rate was recorded every day using a flow meter (Cepex, Spain), while the effluent flowrate was estimated considering the water evaporation losses in the RWs, which were recorded in a local meteorological station at "Estación Experimental Las Palmerillas". The number of daily sun hours was obtained from on-site solar irradiation measurements, while the average external temperature was also recorded at the local meteorological station of "Estación Experimental Las Palmerillas".

2.4 Primary domestic wastewater and CO_2 sources

Primary domestic wastewater was twice per week transported from El Ejido WWTP to the experimental facility (Las Palmerillas, Almería, Spain) and stored in a feed tank of 5,000 L, where it was periodically stirred in order to avoid suspended solids deposition. The wastewater chemical oxygen demand (COD) variations during storage were below 15%. Primary domestic wastewater was subjected to the typical variations of the receiving wastewater in a WWTP in terms of COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, total phosphorus (TP) and *Escherichia coli* (Table 2). Pure CO_2 (99.9%) was purchased from Abello Linde

(Spain), while flue gas (10% CO_2) was obtained on-site from a diesel heating boiler (Tradesa, MOD SF 20, RA GTI, TRADE, Italy).

<Table 2>

2.5 Sampling procedure and calculations

Liquid samples of 200 mL were daily drawn from the mixed liquor of the RWs to determine the TSS concentration, F_v/F_m and culture absorbance at 680 nm. The characterization of the steady states (during operation at constant TSS concentrations, F_v/F_m and culture absorbances at 680 nm) in each RW was carried out during two consecutive sampling days. Fv/Fm was measured with an Aquapen AP 100 fluorometer (Photon Systems Instruments; Czech Republic), while culture absorbance was determined in a double beam Helios spectrophotometer (Spain). The 200 mL mixed liquor samples were filtered through 0.20 μm (Millipore, Spain) in order to simulate biomass harvesting from the RWs with a membrane module. The concentration of COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and total phosphorus (TP) were determined in the above mentioned filtered cultivation medium of each RW and in 100 mL liquid samples of primary domestic wastewater drawn from the stirred storage tank. Similarly, 2 mL of the primary domestic wastewater and of the mixed liquor from each RW were seeded into Petri dishes to determine the total concentration of *Escherichia coli* (*Mc. Conkey* nutrient agar, Scharlau, Spain). The absorption in the visible range (400-800 nm) to determine the biomass extinction coefficient (K_a) was also measured during steady state using 3 mL of mixed liquor samples. Biomass was harvested by centrifugation (Digicen 20, Ortoalresa, Spain) for 5 minutes at 5,000 rpm, resuspended in de-ionized water and centrifuged again in order to wash salts prior to lyophilization (Cuddon, New Zealand).

The elemental (C, N and P) and macroscopic (lipids, proteins, carbohydrates and ashes) biomass compositions were also determined at each steady state.

Process performance was characterized by the steady state removal efficiency (RE) of COD, TOC, TC, TN, N-NH_4^+, TP and *E. Coli*, the mass of CO_2 transferred ($C_{transferred}$) from the gas to the liquid phase, the mass balances of C, N and P, the areal biomass productivity (W) and the biomass extinction coefficient (K_a). The calculation procedures for the above referred parameters are detailed in the supplementary materials section.

2.6 Analytical procedures

COD, TC (TOC+IC) and TN concentrations were determined using Hack-Lange (Germany) kits (LCI 400, LCKI 381 and 238, respectively). TSS, N-NH_4^+, N-NO_3^- and TP concentrations were determined according to the standard methods approved by the Spanish Minister of Agriculture [22]. *Escherichia Coli* was determined according to UNE-EN-ISO 9308-1:2001 [23]. The determination of the C and N content of the algal-bacterial biomass was performed using a LECO CHNS-932 analyzer according to the internal procedure of the University of Almería, while phosphorus content analysis was carried out spectrophotometrically after acid digestion in a microwave according to the internal procedure of the Instrumental Technical Laboratory of the University of Valladolid. Lipids were determined gravimetrically from an extract obtained with 10 mL of a solvent mixture of chloroform:methanol (2:1) (v/v) and 100 mg of dry biomass [24]. The protein content was determined using the Lowry method in dry biomass aliquots of 20 mg [25]. Carbohydrate composition was estimated by the difference between lipids and proteins in the biomass [26]. Finally, total ash content was

determined by incineration at 570ºC for 5 h using 0.5 g sample in an oven (Forns Hobersal, Spain).

3. Results and discussion

3.1 Daily fluctuations of environmental parameters and CO_2 addition in the raceways

The daily temperature and DO variations in the RWs were correlated with the diurnal solar radiation cycle, regardless of the raceway configuration and operational conditions (Fig. 2; Fig. 3). The average light irradiation, ambient temperature, number of daily sun hours and evaporation rates decreased throughout the four experimental stages from 468±292 to 300±157 μmol m^{-2} s^{-1}, from 23±1 to 13±1ºC, from 11 to 7 h and from 6.4±1.8 to 2.9±1.4 L m^{-2} d^{-1}, respectively (Table 3).These variations will inherently occur in any outdoors experimentation and impact the performance of the HRAPs.

<Table 3> <Figure 2> <Figure 3>

This deterioration in the environmental conditions resulted in significant decrease in the average temperatures in the mixed liquors of RW1, RW2 and RW3 from 22.5±4.6, 23.8±4.4 and 22.3±4.3 ºC, respectively, during stage I, to 11.6±3.2, 11.0±3.0 and 9.8±3.0 ºC, respectively, during stage IV (Table 3; Fig.2, Fig. 3). Despite optimum temperatures for microalgae growth often range from 20 to 30ºC, the successful carbon and nutrient removals from piggery and urban wastewaters recorded in similar RWs of 470 L at average mixed liquor temperatures of 7 and 11ºC, respectively, suggests that the low temperatures recorded during this experimentation in stages III and IV would still allow an efficient microalgae-bacterial wastewater treatment [2, 12]. Similarly, maximum DO saturation concentrations of 330, 197 and 234 % were recorded during stage I in RW1, RW2 and RW3, respectively, while the minimum DO saturation

concentrations (26, 85 and 61%, respectively) were recorded during stage IV at night (Fig. 2; Fig. 3). Thus, an O_2-mediated microalgae inhibition could have occurred in stage I in the raceways during peak radiation hours [21], while aerobic conditions always prevailed during the night in all RWs regardless of the operational stage [27]. It is worth noticing that both the highest and lowest DO concentrations were observed in RW1 likely due to the low volumetric mass transfer coefficients in this RW derived from the absence of sump [11].

Likewise, during stage I pH was successfully controlled via pure CO_2 addition at 8.6±0.4, 7.9±0.1 and 7.0±0.1 in RW1, RW2 and RW3, respectively (Fig. 2). Similarly, pH was efficiently controlled via flue gas addition in RW1 and RW2 during stage II (8.4±0.4 and 7.9±0.1, respectively), while flue gas sparging at 20 L min^{-1} was not enough to maintain pH values below 7 during the peak radiation hours in RW3 (7.3±0.3) (Fig. 2f). Under these conditions, the total CO_2 mass transfer rates to the RWs were 5.5, 35.7 and 204.2 mg L^{-1} d^{-1}, which corresponded to CO_2 mass transfer efficiencies to the mixed liquor in RW1, RW2 and RW3 of 6, 31 and 58%, respectively. During stage III, pH values were successfully controlled at 8.0±0.1, 7.9±0.1 and 7.9±0.1 via flue gas injection. This required CO_2 transfer rates of 25.5, 29.1 and 28.1 mg L^{-1} d^{-1}, which represented CO_2 mass transfer efficiencies of 8.5, 52 and 38% in RW1, RW2 and RW3, respectively. Overall, higher CO_2 inputs were required at lower pHs and a lower CO_2 mass transfer efficiency was recorded in the RW without sump (RW1) [11]. Likewise, the low CO_2 mass transfer efficiencies showed that bubble residence times in the RWs were insufficient for complete CO_2 absorption from flue gas with the consequent decarbonation through the water channels. These results were in agreement with those reported by Tredici, [28] and De Godos et al. [20]. Finally, despite not being controlled during stage IV, the pH in the mixed liquor of RW1, RW2 and RW3

averaged 8.5±0.4, 8.3±0.3 and 8.4±0.4, respectively, and was correlated to light irradiation conditions (Fig. 3d, 3e, 3f).

3.2 Wastewater treatment

3.2.1 Influence of pHs and CO_2 source

The COD-REs achieved during stage I using pure CO_2 to control the pH in RW1, RW2 and RW3 were, respectively, 88±1, 88±0 and 81±1%, which were comparable to the COD-REs of 91±3, 88±4 and 92±1% recorded during stage II using CO_2 flue gas (Fig. 4a). The COD effluent concentrations remained lower than 125 mg O_2 L^{-1} regardless of the raceway and operational stage (Table 2), which corresponds to the maximum COD concentration established for wastewater discharge into the environment according to Directive 98/15/CEE [29]. Likewise, TOC-REs during stage I in RW1, RW2 and RW3 were, respectively, 71±0, 73±0 and 68±0%, which were slightly lower than the TOC-REs of 85±1, 83±1 and 83±2% achieved during stage II (Fig. 4b). Thus, while the almost negligible differences between COD and TOC-REs at pH 7, 8 and 9 suggest a minor influence of pH on organic matter removal from wastewater, the slightly superior efficiencies when using flue gas instead of pure CO_2 to control the cultivation pH showed the advantages of this residual CO_2 source in microalgae-based wastewater treatment [14]. It must be highlighted that the environmental conditions in stage I and II did not vary significantly, which allowed a fair comparison of the influence of the source of CO_2. A carbon mass balance was carried out only in stage II due to the above mentioned technical limitations of the GMT 220 CO_2 analyser to measure high CO_2 concentrations. The mass balance calculation revealed that 39, 45 and 37% of the total carbon removed from the wastewater and flue gas in RW1, RW2 and RW3,

respectively, was recovered in the harvested biomass, with similar IC concentrations regardless of the RW in stages I and II (Table 2).

<Figure 4>

TN-REs in RW1, RW2 and RW3 accounted, respectively, for 69±2, 73±1 and 65±1% during stage I, and for 60±6, 75±3 and 62±6% during stage II (Fig. 4c). This corresponded to specific TN removal rates of 44±6, 41±6 and 48±2 mg TN $gTSS^{-1}$ d^{-1} during stage I and 28±5, 29±3 and 26±5 mg TN $gTSS^{-1}$ d^{-1} during stage II, respectively (Table S1, Supplementary Data). The maximum concentration of TN permissible for wastewater discharge into the environment according to Directive 98/15/CEE [29] (15 mg N L^{-1}) was achieved only in RW2 during stage II (Table 2). A N mass balance revealed that 81, 85 and 68% of the TN removed from the wastewater in stage I, and 74, 61 and 60% during stage II was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. N-NH_4^+- REs in stages I and II were higher than 93% in the three RWs evaluated (Fig. 4d). Despite higher N-NH_4^+ volatilizations would be expected at higher pHs ($NH_3 + H^+ \leftrightarrow NH_4^+$; pKa=9.25), N-$NH_4^+$ was rapidly oxidized, which prevented N-NH_4^+ stripping in all RWs [5]. In this context, the high DO and IC concentrations in the mixed liquors, and moderate temperatures, supported an active N-NH_4^+ nitrification, with N-NO_3^- effluent concentrations of 22±1, 19±3 and 24±4 mg N-NO_3^- L^{-1} during stage I, and 19±0, 12±1 and 18±1 mg N-NO_3^- L^{-1} during stage II in RW1, RW2 and RW3, respectively (Table 2). These final N-NO_3^- concentrations corresponded to a significantly similar nitrification activity (estimated as the percentage of influent TN nitrified) during stages I (30.0±1.2, 25.7±0.2 and 28.8±8.1%) and II (32.1±0.8, 21.6±1.0 and 31.2±2.7%) in RW1, RW2 and RW3, respectively. Therefore, neither pH nor the CO_2 source exerted a significant effect on TN-REs. On the other hand, TP-REs in RW1, RW2 and RW3 were, respectively, 41±14, 40±2 and 34±6%

during stage I and 61±17, 63±2 and 65±10% during stage II, resulting in final TP effluent concentrations of 4-6 mg L^{-1}, which were far above the EU discharge limit of 2 mg L^{-1} [29] (Fig. 4e). This represented specific TP removal rates of 3±1, 3±0 and 3±0 mg TP $gTSS^{-1}$ d^{-1} during stage I and 5±2, 5±1 and 6±0 mg TP $gTSS^{-1}$ d^{-1}during stage II, respectively (Table S1, Supplementary Data). In this context, while the pH influence on TP-RE was negligible in the tested range, the use of CO_2 from flue gas exhibited a competitive advantage in terms of TP-RE. Flue gas sparging resulted in lower DO concentrations in the mixed liquors as a result of the higher gas flow rates required to achieve the set pH values (valves were opened for longer periods of time with CO_2 addition from flue gas, data not shown). This could have mediated a higher microalgae and bacterial activity due to the absence of inhibitory DO concentrations and a higher CO_2 availability through the sunny hours in stage II, which likely favored the higher average TOC and TP removals. During stages I and II, 99±1, 95±4 and 95±2% of the TP removed from the wastewater was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. Therefore, assimilation into biomass was the main phosphorus removal mechanism, even in the RW operated at pH 9 (where $P\text{-}PO_4^{3-}$ precipitation would be expected).

E. coli-REs higher than 80% were recorded in all RWs in stages I and II (Fig. 4f). Higher *E. coli* concentrations were observed when decreasing the pH of the mixed liquor (Table 2), which confirmed the positive effect of high pHs in *E. coli* inactivation [30].

3.2.2 Influence of flue gas addition

Based on the results obtained in stage I and II, the RWs were operated at a constant pH of 8 with flue gas CO_2. The increase in the HRT from 2.8±0.2 (stage II) to 6.7±0.4

(stage III) days did not increase the COD-REs, which accounted for 86±3, 87±3 and 88±3%, in RW1, RW2 and RW3, respectively. On the other hand, the absence of pH control during stage IV yielded COD-REs of 73±5, 79±5 and 68±6% in RW1, RW2 and RW3, respectively (Fig. 4a), although this deterioration in the treatment performance was likely due to the less favorable environmental conditions prevailing in stage IV. The similar environmental conditions in stages III and IV allowed for a fair comparison of the influence of pH. COD concentrations in the effluent of the RWs during stages III and IV remained always below the admissible levels for wastewater disposal into the environment, except in RW3 in stage IV (148 mg O_2 L^{-1}) (Table 1). TOC-REs of 72±2, 74±9 and 75±0% were recorded in stage III in RW1, RW2 and RW3, respectively, which slightly decreased to 60±10, 56±9 and 58±7% in stage IV (Fig. 4b). These results confirmed the low influence of pH on organic matter removal and the limited process performance of the three RWs during stage IV as a result of the lower DO concentrations in the mixed liquor mediated by the lower irradiances, temperatures and number of daily sun hours (Fig. 3d, 3e, 3f). During stage III, 87, 67 and 73% of the total carbon removed from the wastewater and flue gas was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. These percentages were considerably higher than in stage II. The C mass balance calculation also revealed that the relative contribution of carbon stripping in RW1, RW2 and RW3 was 13, 5 and 10 times lower in stage III than in stage II, as a result of the lower carbon loads supplied at higher HRTs (Table 2). The higher carbon recovery in RW1 during stage III was likely due to the low CO_2 mass transfer efficiency from the flue gas mediated by the absence of sump, which boosted the depletion of the carbon initially present in the wastewater as a result of algal-bacterial biomass growth. During stage IV, 79, 77 and 84% of the total carbon removed from the wastewater was recovered in the harvested biomass in RW1,

RW2 and RW3, respectively. Despite no CO_2 was added to the RWs during stage IV, IC concentrations in the effluent of the RWs remained similar to those recorded in stage III (Table 2).

The increase in HRT in stage III brought about an increase in TN-REs up to 83±0, 93±2 and 81±3% in RW1, RW2 and RW3, respectively, while operation without pH control in stage IV yielded TN-REs of 97±0, 98±2 and 97±0%. This corresponded to specific TN removal rates of 21±4, 19±6 and 17±4 mg TN $gTSS^{-1}$ d^{-1} during stage III and 32±8, 37±3 and 33±7 mg TN $gTSS^{-1}$ d^{-1} during stage IV, respectively (Table S1, Supplementary Data). This efficient nitrogen removal resulted into final TN concentrations below discharge limits (except in RW1 and RW2 in stage III where TN of 16 and 17 mg L^{-1}, respectively, were recorded) (Fig. 4c, Table 2). The harvested biomass in stage III in RW1, RW2 and RW3, accounted respectively for 48, 52 and 49% of the TN removed from the wastewaters, while the recovered nitrogen as biomass during stage IV was 35, 31 and 34%. N-NH_4^+-REs averaged 86±7% during stage III and 98±0% during stage IV in the three RWs (Fig. 4d). The low temperatures prevailing during the last two operational stages likely caused the wash-out of nitrifying bacteria and consequently no nitrate was detected in these stages. Thus, N-NH_4^+ stripping accounted for most TN removal in the absence of nitrification (stages III and IV), since nitrification contributed to nitrogen sequestration in the previous cultivation stages. These results were in agreement with those reported by García et al. [12], who observed an average contribution of N-NH_4^+ stripping to TN-RE of 32-47% in two HRAPs of 470 L at HRTs of 3-10 d during the treatment of domestic wastewater at outdoor conditions. Similarly, Posadas et al. [5] found a TN-RE decrease from 80 to 60% when nitrification in a 31 L indoor algal turf scrubber photobioreactor treating diluted centrates increased from 9 to 43% at 10.4±0.1 d of HRT. On the other hand, TP-REs during stage III

remained at 64±4, 68±5 and 71±3%, and at 62±1, 61±1 and 56±1% in stage IV in RW1, RW2 and RW3, respectively (Fig. 4e). This represented specific TP removal rates of 2±0, 2±0 and 2±0 mg TP $gTSS^{-1}$ d^{-1} during stage III and 3±0, 3±0 and 3±0 mg TP $gTSS^{-1}$ d^{-1} during stage IV, respectively (Table S1, Supplementary Data). Despite the superior TP-REs mediated by the increase in HRT, TP effluent concentrations still remained above EU regulatory discharge limits (3-4 mg L^{-1}) during stages III and IV (Table 2). The evaluation of P mass balance revealed that 95±5, 90±5 and 86±1% of the TP removed from the wastewater in RW1, RW2 and RW3, respectively, was recovered in the harvested biomass in the last two operational stages, which confirmed that assimilation into biomass was the main TP removal mechanism despite the increase in HRT or the absence of pH control.

Finally, *E. coli*-REs during stage III were slightly higher to those recorded during stage II, and accounted for 97, 75 and 98% in RW1, RW2 and RW3, respectively (Fig. 4f). Stage IV supported the highest *E. coli*-REs (≈99% in the three RWs) among the four stages, which was likely the result of the increase in HRT and the moderately high pH prevailing in the RWs.

3.3 Biomass productivity and characteristics

3.3.1 Influence of pHs and CO_2 source

No influence of the source of CO_2 on biomass productivity was recorded. Hence, areal productivities using pure CO_2 accounted for 13±1, 17±1 and 14±1 g m^{-2} d^{-1} in RW1, RW2 and RW3, respectively, and for 12±1, 13±1 and 14±1 g m^{-2} d^{-1} using flue gas (Fig. 5). These productivities were in agreement with the reported biomass productivity range in outdoors pilot-industrial RWs (10-35 g m^{-2} d^{-1} [31]). However, the TSS concentrations in the three RWs recorded along the four operational stages (321-494 mg

L^{-1}) were low compared to those observed in RWs treating agro-industrial wastewater in previous works. For instance, De Godos et al. [2] reported maximum biomass concentrations of 1,500 mg TSS L^{-1} in a 464 L RW treating piggery wastewater at 10 d of HRT, pH 8, 15ºC and 167 W m^{-2}. Similarly, Posadas et al. [32] recorded maximum biomass concentrations of 2,000 mg TSS L^{-1} in a 180 L RW treating fish farm wastewater diluted with urban wastewater at 7 d of HRT, pH 8.7, 13ºC and 195 W m^{-2}. This showed the high influence of the nature of the treated wastewater on the biomass concentrations in the RW mixed liquor, and consequently on biomass productivity. The slightly higher TSS concentration in RW2 during stages I and II (when environmental conditions remained similar), which also resulted in lower extinction coefficients compared to RW1 and RW3 (0.08-0.12 m^2 g^{-1} compared to 0.15-0.25 m^2 g^{-1}) (Table 4), suggested a favored algal-bacterial biomass growth at pH 8. The quantum yield in stages I and II remained constant at 0.34±0.01 (Table 4), which were low compared to typical reported yields of 0.75 in synthetic mineral salt medium [33] but similar to the quantum yields (0.38) in domestic wastewater [26].

<Figure 5>

3.3.2 Influence of flue gas addition

The increase in HRT brought about a significant decrease in biomass productivities compared to stage II, which accounted for 4±0, 4±1 and 5±1 g m^{-2} d^{-1} in RW1, RW2 and RW3, respectively. Based on the similar TSS concentrations regardless of the operational stage and RW (Table 4), it can be concluded that the HRT strongly influenced biomass productivity. On the other hand, process operation in the absence of pH control supported biomass productivities of 7±0, 5±1, 6±1 g m^{-2} d^{-1} during stage IV (Fig. 5). These results clearly showed that CO_2 addition from flue gas did not result in a biomass productivity increase under operation at high HRT. At these operational

conditions, the extinction coefficient ranged from 0.13 to 0.20 m^2 g^{-1} (Table 4). These K_a values were relatively low compared to the minimum of 0.19 m^2 g^{-1} recorded when secondary wastewater was treated in 250 mL photobioreactors [26], which was likely induced by the high solid concentration (1,700 mg L^{-1}) supported by this particular photobioreactor lab scale configuration. The average quantum yield, Fv/Fm, during stages III and IV were 0.53±0.07 and 0.47±0.04, respectively (Table 4). These higher quantum yields compared to stages I and II at lower irradiances, temperatures and sun hours suggested a possible microalgae activity increase at low irradiances. Similar results were reported by Vonshak and Torzillo [34], who found a reduction of 30% in the quantum yield when irradiance increased from 167 to 750 W m^{-2} at 25ºC in outdoors tubular photobioreactors.

3.4 Biomass composition

3.4.1 Influence of pHs and CO_2 source

A higher C, N and P content was observed in the biomass when flue gas (stage II) was sparged into the mixed liquors regardless of the pH (Table 5). The highest carbon content was recorded at pH 8 (43.9 and 61.5% in stages I and II, respectively) and the lowest N and P contents at pH 7 (N: 6.4 and 9.5% and P: 1.1 and 2.0% during stages I and II, respectively). No influence of the pH on the macromolecular composition (in terms of lipid, protein and carbohydrate content) of the biomass generated along stages I and II was recorded (Table 5). In this context, the protein content remained constant during the four operational stages (≈38±3%), resulting in a constant Protein/N ratio of 4.1±0.5, in agreement with the 4.4 ratio reported by González-López et al. [35]. The biomass lipid and carbohydrates contents exhibited the largest variation with operational conditions. Thus, while the supply of pure CO_2 supported lipid contents of

6.0, 5.8 and 7.2% in RW1, RW2 and RW3, respectively, CO_2 addition from flue gas unexpectedly increased the lipid content up to 23.0, 18.4 and 16.7% in RW1, RW2 and RW3, respectively. Conversely, carbohydrate accumulation was favored by the supply of pure CO_2. In this context, lipid synthesis by microalgae cells could have been influenced by the higher CO_2 availability when using flue gas as a result of its more homogeneous supply. Despite the reasonably high biomass productivity and the highest lipid content during stage II, the resulting biodiesel productivities are not profitable for biodiesel production with the current cost of fossil fuels [6].

<Table 5>

3.3.2 Influence of flue gas addition

The increase in HRT led to higher C biomass contents in RW1 and RW3 (64.8 and 61.6%, respectively), and in similar N and P content (≈10% N and ≈2% P) (Table 5). The impact of the decrease in temperature from stage II to stage III on C biomass content can't be however ruled out, but these variations are inherent to outdoors experimentation at semi-industrial scale. On the other hand, process operation in absence of CO_2 from flue gas sparging (stage IV) resulted in a significant decrease in the C, N and P biomass content regardless of the RW (Table 5). These results were in agreement to the empirical C compositions reported by Arbid et al. [16], who respectively recorded a C content increase from 40.2±1.5 and 40.0±1.0% to 43.5±1.8 and 42.5±1.6% in HRAPs with and without sump at 8 d of HRT under outdoors conditions during the treatment of domestic wastewater. Overall, flue gas sparging in stages II and III during wastewater treatment supported the highest carbon biomass content compared to process operation with addition of pure CO_2 or in the absence of pH control (Table 5). Likewise, the highest nitrogen and phosphorus biomass contents

were also recorded during the process operation with flue gas addition (II and III) regardless of the pH. The C/N and N/P ratios of the harvested biomass throughout the four operational stages were both 6±1, which highlighted the consistent chemical composition of the algal-bacterial biomass generated despite the changes in operational conditions. The constant C/lipid of 4.3±1 in stages III and IV showed that flue gas sparging did not impact lipid synthesis under these operational conditions. This fact could have been caused by the higher influence of other factors such as lower light irradiances, number of sun hours [36] or temperatures (compared to stages I and II) on lipid synthesis.

Conclusions

The influence of pH was negligible in terms of wastewater treatment performance, while CO_2 sparging from flue gas instead of pure CO_2 supported slightly higher COD and TOC-REs, and significantly higher TP-REs. On the other hand, CO_2 addition from flue gas compared to process operation without CO_2 supplementation contributed to pH control but did not improve wastewater treatment performance or biomass productivity as a result of the intensive CO_2 stripping from the RW mixed liquor. Finally, biomass C, N and P content, and macroscopic composition, were significantly impacted (except for protein) by the nature of the supplemented CO_2. Overall, flue gas sparging for pH control was shown the most effective and environmentally friendly alternative for RWs operation due to its contribution to greenhouse gas emission mitigation concomitantly with wastewater treatment and production of a valuable microalgae biomass.

ACKNOWLEDGMENTS

This study was supported by Aqualia as a part of the project ITACA. The practical assistance of the staff of the Estación Experimental Las Palmerillas from Fundación

Cajamar is gratefully acknowledged. Finally, Valladolid University is also gratefully acknowledged for funding doctoral contract of Esther Posadas (project Ref. GR76/RTA2013-00056-C03-02/ CONSOLIDER-CSD 2007-00055).

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FIGURE CAPTIONS

Figure 1. a) Schematic of the three raceway photobioreactors. White circles in the RWs represent pH sensor, while grey circles refer to the sensors of dissolved oxygen, temperature, and CO_2 composition. Continuous and discontinuous lines indicate domestic wastewater and CO_2 distribution, respectively. b) Schematic of a raceway with common dimensions, paddlewheel and sump (black circle).

Figure 2. Daily time course of DO (–◇–), temperature (–■–), pH (–●–) and light radiation (Ra) (–△–) during stage I in RW1 (a), RW2 (b) and RW3 (c), and stage II in RW1(d), RW2 (e) and RW3 (f) under steady state operation.

Figure 3. Daily time course of DO (–◇–), temperature (–■–), pH (–●–) and light radiation (Ra) (–△–) during stage III in RW1 (a), RW2 (b) and RW3 (c), and stage IV in RW1 (d), RW2 (e) and RW3 (f).

Figure 4. Removal efficiency of (a) COD, (b) TOC, (c) TN, (d) $N\text{-}NH_4^+$, (e) $P\text{-}PO_4^{3-}$, and (f) *Escherichia coli* in RW1 (□), RW2 (■) and RW3 (■) during the steady state of the four operational stages.

Figure 5. Biomass productivity in the mixed liquor of RW1 (□), RW2 (■) and RW3 (■) during the four operational stages.