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Microalgae recycling improves biomass recovery from wastewater treatment high rate algal ponds

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Abstract

Microalgal biomass harvesting by inducing spontaneous flocculation (bioflocculation) sets an attractive approach, since neither chemicals nor energy are needed. Indeed, bioflocculation may be promoted by recycling part of the harvested microalgal biomass to the photobioreactor in order to increase the predominance of rapidly settling microalgae species. The aim of the present study was to improve the recovery of microalgal biomass produced in wastewater treatment high rate algal ponds (HRAPs) by recycling part of the harvested microalgal biomass. The recirculation of 2% and 10% (dry weight) of the HRAPs microalgal biomass was tested over one year in an experimental HRAP treating real urban wastewater. Results indicated that biomass recycling had a positive effect on the harvesting efficiency, obtaining higher biomass recovery in the HRAP with recycling (R-HRAP) (92-94%) than in the control HRAP without recycling (C-HRAP) (75-89%). Microalgal biomass production was similar in both systems, ranging between 3.3 and 25.8 g TSS/m^2d, depending on the weather conditions. Concerning the microalgae species, *Chlorella* sp. was dominant overall the experimental period in both HRAPs (abundance >60%). However, when the recycling rate was increased to 10%, *Chlorella* sp. dominance decreased from 97.6 to 88.1%; while increasing the abundance of rapidly settling species such as *Stigeoclonium* sp. (16.8%, only present in the HRAP with biomass recycling) and diatoms (from 0.7 to 7.3%). Concerning the secondary treatment of the HRAPs, high removals of COD (80%) and N-NH_4^+ (97%) were found in both HRAPs. Moreover, by increasing the biomass recovery in the R-HRAP the effluent total suspended solids (TSS) concentration was decreased to less than 35 mg/L, meeting effluent quality requirements for discharge. This study shows that microalgal biomass recycling (10% dry weight) increases biomass recovery up to 94% by selecting the most rapidly settling microalgae species without compromising the biomass production and improving the wastewater treatment in terms of TSS removal.

Keywords:

Bioflocculation; biomass recovery; microalgae species selection; microalgal biomass production;

harvesting

1. Introduction

In recent years, much attention has been paid to microalgae-based systems for wastewater treatment and biomass production like high rate algal ponds (HRAPs). In fact, microalgal biomass grown as a by-product of wastewater treatment is nowadays considered as a cost-effective feedstock for bioenergy production. Despite bioenergy production from microalgae has well-known advantages in front of other biomass sources (i.e. fast growth rates and lack of competence for agricultural land or water), each step of the process from microalgae production to bioenergy conversion still has to be improved in order to reduce the operating costs of the entire process (Mehrabadi et al., 2015).

Specifically, current biomass harvesting techniques increase the cost of microalgae production, representing about 20-30% of the total cost (Molina-Grima et al., 2003; Zittelli et al., 2006). Recently, life cycle assessments and cost analysis of different harvesting techniques have been conducted to assess the cost-efficiency and environmental impact of the most common harvesting techniques (Udom et al., 2013). Methods commonly employed include the addition of chemicals or the use of mechanical equipment that increase costs (e.g. flocculation induced by chemical addition, filtration, centrifugation, sonication, electro-flocculation). In wastewater treatment, gravity sedimentation is the most common solids separation method, used to clarify large volumes of treated wastewater with reasonable costs (<5% of the total cost) (Metcalf and Eddy, 2003). The biomass grown in HRAPs for wastewater treatment is constituted by mixed populations of microalgae and bacteria which form spontaneous flocs (diameter 50-200 μm) that can partially settle by gravity without chemicals or energy addition (García et al., 2000; Park et al., 2011a; Valigore et al., 2012). Indeed, inside these flocs, microorganisms interaction provides natural occurring processes inducing their spontaneous flocculation (Salim et al., 2011; Golueke and Oswald, 1970).

For these reasons, in the last years a niche of research of harvesting techniques has focused on the optimization of spontaneous flocculation and gravity sedimentation (Van Den Hende et al., 2011; González-Fernández et al., 2013). Different methods and strategies to improve microalgal

harvesting have shown promising results regarding spontaneous flocculation. Some of these methods are the coprecipitation with ions at high pH (autoflocculation) and release of extracellular polymeric substances, or microalgae-bacteria interaction (bioflocculation) (González-Fernández et al., 2013, Wan et al., 2014). A recently developed strategy consists in promoting the dominance of rapidly settling microalgae species by recycling a small part of the biomass harvested in gravity settlers (Park et al., 2011b). Thus, species that can settle easily are selected competitively against poorly settling species.

Following this promising approach, the aim of the present study was to enhance microalgal biomass harvesting efficiency by recycling an increasing amount of harvested biomass and to determine its effect on biomass production, microalgae species evolution and wastewater treatment performance. Recycling rates of 2% and 10% (dry weight) of the microalgal biomass grown in the HRAPs were tested in order to improve the spontaneous flocculation of algae-bacteria biomass in experimental HRAPs treating real urban wastewater. Harvesting efficiency results were evaluated in terms of biomass recovery and microalgal biomass settling velocities distribution.

2. Material and Methods

2.1 Experimental microalgae-based wastewater treatment system

Two experimental HRAPs located outdoors at the facilities of the Environmental Engineering and Microbiology Research Group (GEMMA) of the Universitat Politècnica de Catalunya·BarcelonaTech (Barcelona, Spain) were used. These HRAPs were continuously operated since 2010 (Passos et al., 2015). For the purpose of this research, the HRAPs were monitored over one year (from March 2014 to March 2015). Raw urban wastewater from a nearby municipal sewer was daily pumped to a homogenisation tank (volume of 1.2 m^3) and uninterruptedly pumped to a primary settler with a useful volume of 7 L, a surface area of 0.0255 m^2 and a hydraulic retention time (HRT) in the range of 0.7-1.4 h. The primary settler effluent (from now on referred to as primary effluent) was discharged into both HRAPs by means of two peristaltic pumps. Both HRAPs

operated at the same HRT during the whole experimental period. As suggested by García et al. (2000), the theoretical HRT was modified over the year (8, 6 and 4 days) by regulating the flow rates (120, 78.5 and 60 L/d for 4, 6 and 8 days of HRT, respectively) in accordance with the weather conditions (i.e. solar radiation and temperature). In fact, these systems require longer HRT in cold weather conditions with low solar radiation in order to accomplish wastewater treatment and meet effluent quality requirements for discharge.

Each HRAP, built in PVC, had a surface area of 1.54 m^2, 0.3 m of water depth and a useful volume of 0.47 m^3. Continuous stirring of the mixed liquor avoided biomass sedimentation and assured microalgae contact with sunlight. This was achieved by means of two paddle-wheels driven by an engine (5 rpm) reaching a flow velocity of 10 cm/s in the mixed liquor. Biomass growing in the HRAPs was harvested in two secondary settlers (one per each HRAP) with a useful volume of 3.1 L, a surface area of 0.013 m^2 and a critical settling velocity of 0.4, 0.25 and 0.2 m/h (HRT of 0.6, 1 and 1.2 h, respectively) depending on the HRT of the HRAPs. Around 1-1.5 L of harvested biomass with a total solids concentration between 1-2% (w/w) (depending on the period of the year) were purged from each settler every weekday.

2.2 Biomass recycling

In order to evaluate the influence of biomass recycling on the harvesting efficiency, microalgal biomass production and wastewater treatment, biomass recycling was set-up in one HRAP, while the other one was used as a control (from now on referred to as R-HRAP and C-HRAP, respectively). Figure 1 shows a schematic diagram of the process in the R-HRAP line. In a previous study by Park et al. (2011b), a constant volume of 1 L of harvested microalgal biomass was daily recycled to a 8 m^3 HRAP. In this previous study, the constant recirculation volume applied did not take into account the variation of the solids concentration in the HRAP mixed liquor. From the data presented by Park et al. (2011b), a recycling rate between 2-16% (dry weight) of the HRAP microalgal biomass was inferred. Taking this range of values as reference, two different recycling

rates (2% and 10% dry weight) were tested in the present study, corresponding to a variable recycling flow rate. The recycling flow rate was calculated weekly following Eq. (1).

$$V_R = \frac{Recyclingrate*(TSS_{HRAP}*V)}{TSS_{settler}}$$

Eq. (1)

Where V_R is the volume recycled daily (L); TSS_{HRAP} is the mixed liquor total suspended solids concentration inside the HRAP (mg/L); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and V is the HRAP volume (L).

Due to biomass recycling, in the R-HRAP the solids retention time (SRT) was higher than the HRT, while the SRT and HRT were identical in the C-HRAP. The SRT of the R-HRAP was calculated by Eq. (2) according to Metcalf and Eddy (2003).

$$SRT = \frac{V * TSS_{HRAP}}{(Q - Q_E + Q_P) * TSS_{HRAP} - Q_R * TSS_{Settler}}$$

Eq. (2)

Where Q is the primary effluent flow rate (L/d); Q_E is the evaporation rate (L/d) and Q_P is the precipitation rate (L/d); Q_R is the recycled flow rate (L/d); TSS_{HRAP} is the mixed liquor total suspended solids concentration inside the HRAP (mg/L); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and V is the total volume of the HRAP (L).

The evaporation rate was calculated following Eq. 3.

$$Q_E = \frac{E_p A}{7}$$

Eq. (3)

Where A is the surface area of the HRAP (m^3) and E_p is the potential evaporation between weekly samples (mm) which was calculated from Turc's formula (Eq. 4).

$$E_p = a(R + 50)\frac{T}{T + 15}$$

Eq. (4)

where R is the average solar radiation in a week (cal/cm^{2}d); T is the average temperature in a week (ºC); *a* is the dimensionless coefficient which varies depending on the time between samples. The value of *a* for weekly samples is 0.091.

Solar radiation, air temperature and precipitation data were obtained from a nearby meteorological station (Department of Astronomy and Meteorology, University of Barcelona, http://infomet.am.ub.es).

To evaluate the biomass harvesting efficiency, the biomass recovery (%) was calculated following Eq. 5.

$$Biomassrecovery(\quad) = \frac{TSS_{HRAP} - TSS_{Effluent}}{TSS_{HRAP}} * 100 \qquad \text{Eq. (5)}$$

Where TSS_{HRAP} is the mixed liquor total suspended solids concentration inside the HRAP (mg/L) and $TSS_{Effluent}$ is the total suspended solids concentration of the secondary settler effluent (mg/L).

The experiment was divided into four periods characterised by different HRTs (depending on the season) and recycling rates: period 1 (HRT: 8 days, recycling rate: 2%), period 2 (HRT: 4 days, recycling rate: 2%), period 3 (HRT: 6 days, recycling rate: 10%) and period 4 (HRT: 8 days, recycling rate: 10%). The main operational parameters and primary effluent characteristics of the HRAP systems with and without recycling are summarised in Table 1.

2.3 Settling velocities distribution of microalgal biomass

The settling velocities distribution of microalgal biomass from both HRAPs was studied by means of a dynamic sedimentation test using a water elutriation apparatus. In this device biomass flocs are washed out according to their relative density, volume and form, under dynamic conditions. Microalgal biomass passes through three settling columns with increasing diameters (50 mm, 100 mm and 200 mm of nominal diameter for C1, C2 and C3 settling columns, respectively) interconnected in series. For each test, 25 L of HRAP mixed liquor were poured to a continuously stirred inlet tank (30 L) and then pumped through the columns. The flow rate in these tests was set

to 0.21 L/min in order to have a range of critical settling velocities (0.4 - 6.5 m/h) similar to those used in secondary settlers (0.7 – 1.3 m/h according to Metcalf and Eddy (2003)). The sample entered each column near the bottom and exited near the top. Note that the critical settling velocity decreased progressively in successive columns due to their gradual increase in column diameter, and therefore biomass flocs were retained in the different columns depending on their settling velocities. Flocs with a settling velocity equal to or higher than the critical settling velocity of a given column were retained in this column, while flocs with a settling velocity lower than the critical settling velocity escaped to the next column. Flocs with a settling velocity lower than the critical velocity of the third column escaped, and were therefore collected in a 30 L outlet tank. Consequently, the first column retained flocs with a settling velocity ≥6.5 m/h, the second one between 6.5 and 1.6 m/h, and the third one between 1.6 and 0.4 m/h. A detailed description of the apparatus and of the method can be found in Gutiérrez et al. (2015) and Gutiérrez et al. (2016).

Four dynamic sedimentation tests (one per HRAP) were conducted: two with samples from period 2 (recycling rate) and two (one per HRAP) with samples from period 4 (recycling rate 10%). At the moment of sedimentation tests in period 2, the total suspended solids concentration were similar for the R-HRAP and C-HRAP (230 mg TSS/L and 240 mg TSS/L, respectively). In contrast, higher differences were observed at the time of sedimentation tests in period 4, with solids concentration of 420 mg TSS/L and 130 mg TSS/L for the R-HRAP and C-HRAP, respectively.

2.4 Biomass production and characterisation

Biomass production was quantified once a week based on the total suspended solids concentration (g TSS/m^{2}d) of the mixed liquor collected from the two HRAPs and determined following Eq (6). Evaporation and precipitation rates were taken into account.

$$Microalgalbiomassproduction = \frac{TSS_{HRAP} \cdot [Q - Q_E + Q_P] - [TSS_{Settler} Q_R]}{A \cdot 1000} \quad \text{Eq. (6)}$$

where TSS_{HRAP} is the total suspended solids concentration of the mixed liquor HRAP (mg TSS/L);

Q is the primary effluent flow rate (L/d); Q_E is the evaporation rate (L/d); Q_P is the precipitation rate (L/d); Q_R is the recycled flow rate (L/d); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and A is the surface area of the HRAP (m^2). The term in brackets with asterisk was only taken into account for the R-HRAP (in this HRAP biomass production was calculated by subtracting the recycled biomass not to overestimate the TSS concentration of the R-HRAP).

To prove the recycling effect on population dynamics, two sampling campaigns were conducted for microorganisms identification. The first campaign was conducted in periods 1 and 2 (2% recycling rate) over 3 months, with 13 samples analysed. The second campaign was carried out in period 4 (10% recycling rate) over 3 months, with 11 samples analyzed. During these campaigns, 250 mL samples were taken once a week from the mixed liquor of the HRAPs. From these samples, microalgae species were identified and quantified. Other co-occurred microorganisms (ciliates and rotifers) were also identified. Microalgae identification was carried out by optic microscope examination (Motic BA310E, China), equipped with a camera (NiKon DS-Fi2) using the software NIS-Elements Viewer. Microalgae *genus* were identified from classical specific literature (Palmer, 1962; Bourelly, 1966). For microalgae quantification, two replicates of 25µL of each well homogenised sample were examined by bright and contrast phase microscopy using a Zeiss microscope Axioskop 40. In each subsample, microalgae were counted *in vivo* at 100 and 400 magnification using coverslides of 20 mm side (Salvadó et al., 2004). Different methods were conducted to quantify microalgae depending on the species. *Stigeoclonium* sp. (filamentous microalgae) were quantified according to the intersection method developed by Salvadó (Salvadó, in press). As for *Chlorella* sp. and diatoms, the aggregated flocs of these unicellular species were broken down by means of an ultrasound technique (Abzazou et al., 2015).

2.5 Wastewater treatment efficiency

Wastewater treatment performance was monitored during the whole year. Samples from the mixed

liquor of the two HRAPs as well as the primary effluent (influent of the HRAP) were weekly collected and analysed for ammonium nitrogen ($N\text{-}NH_4^+$) and chemical oxygen demand (COD) to evaluate the secondary treatment carried out by the HRAPs.

To evaluate the COD removal, samples of the primary effluent were analysed (without filtration) obtaining the total COD (TCOD). On the other hand, samples of the HRAPs mixed liquor were filtrated (glass fiber filters of 47 mm and average pore size 1 μm) in order to avoid the microalgae contribution to the organic matter content, obtaining the soluble COD (SCOD). Total (TCOD) and soluble (SCOD) were analysed according to Standard Methods (APHA-AWWA-WPCF, 2001) and $N\text{-}NH_4^+$ was measured from filtered samples according to the Solorzano method (Solorzano, 1969). All the analyses were undergone in triplicate and results are given as average values.

2.6 Statistical analysis

The effect of biomass recycling on wastewater treatment performance, microalgal biomass production and harvesting efficiency was evaluated by means of the Student's paired t test using Minitab 17.0 software. p=0.05 was set as the level of statistical significance.

3. Results

3.1. Microalgal biomass harvesting

3.1.1. Biomass recovery

Microalgal biomass concentration in the mixed liquor and in the effluent of secondary settlers from both HRAPs, along with the calculated biomass recovery are shown in Figure 2. Mixed liquor biomass concentration from the HRAPs varied over the year between 83-683 mg TSS/L for the R-HRAP and between 47-489 mg TSS/L for the C-HRAP, respectively. Less variability was observed in effluent concentrations, which varied between 8-54 mg TSS/L for the R-HRAP and between 11-63 mg TSS/L for the C-HRAP. Average values of these concentrations and biomass recoveries for each period are summarised in Table 2. The mixed liquor average biomass concentration in the R-

HRAP was higher than in the C-HRAP (30-459 mg TSS/L vs. 144-353 mg TSS/L, respectively). Furthermore, the effluent biomass concentration from the R-HRAP settler (18-30 mg TSS/L) was lower than in the C-HRAP settler (34-54 mg TSS/L), indicating higher average biomass recovery in the R-HRAP (92-94%) than in the C-HRAP (75-89%).

When the recycling rate was 2%, the difference between the biomass recoveries of the R-HRAP and C-HRAP decreased from 14% (period 1) to 4% (period 2) (Table 2). However, when the recycling rate was increased to 10%, the difference between biomass recoveries of both HRAPs increased to 16% (period 4). Statistical analysis reported significant differences between biomass recoveries ($p<0.05$), highlighting the great influence of recycling on the harvesting efficiency of microalgal biomass.

3.1.2. Biomass settling velocities distribution

Two sedimentation tests (one for each HRAP) were carried out in period 2 (Fig. 3a) and period 4 (Fig. 3b) in order to evaluate the effect of biomass recycling on the settling velocities distribution of microalgal biomass. In Figure 3, each pair of bars refers to the microalgal biomass with a certain settling velocity. By adding the percentages of the first two bars, the amount of biomass with settling velocities ≥1.6 m/h was obtained. Results from period 2 (recycling rate: 2%, HRT: 4 days) indicate that 80% of the biomass from the C-HRAP had settling velocities ≥1.6 m/h, while this value was increased to 95% in the case of the R-HRAP (Fig. 3a). This means that the amount of rapidly settling biomass increased when biomass recycling was applied. In period 2, the critical settling velocity of secondary settlers of the pilot plant was 0.4 m/h. Therefore, the amount of biomass recovered with settling velocities > 0.4 m/h (sum of the percentages of the first three bars) obtained in the sedimentation test should be similar to the biomass recovery obtained in the secondary settler (Table 2). When the sedimentation test was conducted (last week of June), biomass recoveries of 98% and 90% were achieved in the secondary settler of the R-HRAP and C-HRAP, respectively. Similarly, the microalgal biomass recovered with settling velocities > 0.4 m/h

in the sedimentation test was 98% and 93% for the R-HRAP and the C-HRAP, respectively, which was close to the biomass recovery of secondary settlers.

On the other hand, sedimentation tests carried out in period 4 (recycling rate: 10%, HRT: 8 days) showed that 86% of the biomass from the R-HRAP had settling velocities > 1.6 m/h, in contrast with only 5% of the biomass from the C-HRAP (Fig. 3b). Considering settling velocities of 0.4 m/h, 36% of the biomass from the C-HRAP was recovered in comparison with 92% of the biomass from the R-HRAP. This explains important differences in biomass recovery (16% in average) found in period 4 when the recycling rate was 10%, as compared to period 2 (2% recycling rate) when the difference between the biomass recoveries from both HRAPs was only 4%.

3.2 Microalgal biomass production and characterization

3.2.1. Microalgal biomass production

Microalgal biomass production in both HRAPs is shown in Figure 4. Average values of biomass production from each period are summarised in Table 2. The biomass production in both HRAPs was not significantly different ($p>0.05$); therefore biomass recycling did not affect biomass production. Seasonal biomass production variations were mostly related to changes in HRT and weather conditions. As expected, higher biomass production was observed in periods with favourable environmental conditions than in periods with adverse conditions. Therefore, in period 2 (summer) a high average biomass production of 25.8 g TSS/m^2d was reached in both HRAPs, while in period 4 (autumn and winter) the average biomass production decreased to 3.3 g TSS/m^2d in the R-HRAP and to 5.5 g TSS/m^2d in the C-HRAP.

Similar results of biomass production were obtained by Park et al. (2011b) who operated an experimental HRAP (8 m^3) treating primary effluent, with recycling rates between 2 and 16% of the HRAP microalgal biomass and CO_2 addition, under similar weather conditions (Hamilton, New Zealand). They reported an annual average biomass production of 9.2 g VSS/ m^2d and 10.9 g VSS/m^2d for the C-HRAP and the R-HRAP, respectively. Considering that in our study the TSS of

313 the HRAPs mixed liquor were predominantly organic (VSS/TSS ratio of 0.8-0.9), a similar biomass
314 production was attained here, reaching an average value of 10.4 g VSS/m^2d (or 13 g TSS/m^2d) in
315 both HRAPs. Except for the last period when the lowest production was registered, during the rest
316 of the year the microalgal biomass production ranged between 10.5 and 25.8 g TSS/m^2d in both
317 HRAPs, falling into the range of 10 – 35 g TSS/m^2d found in outdoor systems dominated by green
318 microalgae (Park and Craggs, 2010; Heubeck et al., 2007).
319
320 ***3.2.2. Microalgal biomass characterization***
321 In this study, the most abundant species identified in both HRAPs was the green microalgae
322 *Chlorella* sp. (Figures 5 and 6). The diatoms *Nitzschia* sp. and *Navicula* sp., and the filamentous
323 green microalgae *Stigeoclonium* sp. were also present. Moreover, microalgae grazers like ciliate and
324 flagellate protozoa were continuously observed.
325 Even if the green unicellular microalgae *Chlorella* sp. was the dominant species over the whole
326 experiment (Figure 6), fluctuations in weather conditions (temperature and solar radiation) together
327 with changes of HRT, led to slight variations in microalgae populations abundance.
328 Concerning the influence of recycling on microalgae populations, in periods 1 and 2 (2% recycling
329 rate) the abundance of *Chlorell*a sp. and diatoms was slightly different in the two HRAPs (Fig. 6a
330 and 6b), resulting in average values of 97.6% and 98.9% for *Chlorella* sp. and 0.74% and 0.84% for
331 diatoms, in the R-HRAP and the C-HRAP, respectively. On the other hand, *Stigeoclonium* sp.
332 abundance in the R-HRAP (1.64%) was slightly higher than in the C-HRAP (0.31%). When the
333 recycling rate was increased from 2% to 10%, higher differences were observed between systems
334 (Fig. 6c and 6d). Average percentages of *Chlorella* sp. of 88% and 96% where observed in the R-
335 HRAP and C-HRAP, respectively. In the same period, average percentages of diatoms of 7.3% and
336 4.1% were found in the R-HRAP and C-HRAP, respectively. Thus, higher recycling rates decreased
337 *Chlorella* sp. in favour of diatoms. Note that diatoms *Nitzschia* sp. and *Navicula* sp. are benthic
338 organisms linked to flocs; therefore their increase indicates a higher amount of flocs. Moreover,

during this period (10% recycling) *Stigeoclonium* sp. was detected only in the R-HRAP, reaching a maximum abundance of 38% at the beginning of period 4.

3.3 Secondary wastewater treatment

Despite selecting different HRT according to the season, a high variability was observed in organic matter removal efficiency in both HRAPs (Fig. 7). This was linked to the high variability of the influent COD concentration over the experiment (100-800 mg O_2/L) (Table 1), which did not seem to affect the effluent concentration (ranging between 50 and 70 mg O_2/L). Besides, a similar organic matter removal was registered in both HRAPs. Altogether, COD removal efficiencies were 59-94% for the R-HRAP and 56-93% for the C-HRAP, with an average COD removal of 80% in both systems along the experiment.

Similar ammonium nitrogen removal was also observed in both HRAPs over the year (Fig. 8). Influent $N\text{-}NH_4^+$ concentrations ranged between 26 and 36 mg $N\text{-}NH_4^+$/L, while effluent concentrations were below 4.7 and 3.8 mg $N\text{-}NH_4^+$/L in the R-HRAP and C-HRAP, respectively (Table 1). In this case, an average $N\text{-}NH_4^+$ removal of 95% was registered in periods 1 and 2 in both HRAPs. Such a good performance was even enhanced in periods 3 and 4 with 99% removal in both HRAPs. Statistical analysis showed that COD and $N\text{-}NH_4^+$ removal efficiencies were not significantly different ($p>0.05$) between the two HRAPs (with $p=0.82$ for COD removal efficiency and $p=0.06$ for $N\text{-}NH_4^+$ removal efficiency). These results are in accordance with those reported by Park et al. (2011b), who obtained similar ammonium nitrogen removals (86-96%) with and without biomass recycling.

In term of TSS concentrations in the HRAPs effluent, recycling had a positive effect due to the higher biomass recovery previously mentioned. Indeed, only the effluent from the R-HRAP settler (18-30 mg TSS/L) met the European urban wastewater treatment 91/271/EEC Directive (Council Directive, 1991); while the C-HRAP (TSS concentrations ranging between 34 and 54 mg TSS/L) always exceeded the limit of 35 mg TSS/L.

4. Discussion

In the present study, a great influence of biomass recycling on the harvesting efficiency of microalgae was observed. When 2% of the HRAP biomass was recycled, the average increase in biomass recovery was only 9%, with the highest abundance of *Chlorella* sp. (around 98% on average). When recycling was increased to 10% of the HRAP biomass, the difference in biomass recovery between the C-HRAP and R-HRAP increased to 17%, corresponding to 1) lower *Chlorella* sp. abundance (74.7% on average), 2) higher abundance of *Stigeoclonium* sp. (up to 38%) and 3) increase of diatoms abundance (from 0.7 to 7.3%) in the R-HRAP. *Stigeoclonium* sp. formed macroscopical thalli in the form of flocs. Hence, the increase of recycling rate improved the biomass recovery by increasing the presence of microalgae capable of forming macroscopical structures (like *Stigeoclonium* sp.) or microalgae linked to flocs (diatoms). Indeed, the presence of microalgae species with these properties, which settled more easily (e.g. *Stigeoclonium* sp.) has been reported to have a significant influence on the harvesting efficiency (Kim et al., 2014; Van Den Hende et al., 2014). In addition, Park et al. (2013a) studied similar systems with biomass recycling and observed that the harvesting efficiency and biomass production were affected by microalgae species selection and increased floc formation. Other studies also pointed out the influence of specific strains on microalgal biomass harvesting efficiency (Gutiérrez et al., 2016; Su et al., 2012).

As stated before, the presence of *Stigeoclonium* sp. (capable of forming macroscopical structures) and diatoms (linked to flocs) led to the formation of larger sized algal colonies and/or algal/bacterial aggregates in the culture, which increased the settling ability of microalgal biomass (Park et al., 2013b). These algal/bacterial aggregates would have a lower surface area to volume ratio, leading to a higher settling velocity. Large microalgal flocs composed by *Stigeoclonium* sp. (around 38% dominance), *Chlorella* sp. and diatoms (>20μm) were identified in the R-HRAP, while less compacted flocs of *Chlorella* sp. and diatoms, and some dispersed cells were observed in the C-HRAP (Fig. 5). From this analysis, it was expected that microalgal biomass from the R-HRAP

would form larger algal/bacterial aggregates resulting in higher biomass settling velocities due to the presence of rapidly settling species. Results from the sedimentation test when 10% of the biomass was recycled confirmed this hypothesis. Indeed, it showed that 86% of the microalgal biomass in the R-HRAP had settling velocities higher than 1.6 m/h when rapidly settling microalgae species (e.g. *Stigeoclonium* sp. and/or diatoms) were identified. In contrast, only 5% of the microalgal biomass in the C-HRAP had settling velocities higher than 1.6 m/h, when poorly settling microalgae (e.g. *Chlorella* sp.) were found (Fig. 3b). Biomass recoveries obtained in secondary settlers (0.2 m/h of settling velocity) in this period were 76% and 92% for the C-HRAP and the R-HRAP, respectively, which was the highest difference between the two systems over the year.

5. Conclusions

This study showed the effect of two recycling rates of HRAP microalgal biomass (2 and 10% dry weight) on the biomass harvesting efficiency, biomass production, microalgae species evolution and secondary wastewater treatment in HRAPs. The following conclusions can be drawn from the results:

- Biomass recycling had a positive effect on the harvesting efficiency enhancing the biomass recovery in the R-HRAP to 92-94% (vs. 75-89% in the C-HRAP). Moreover, recycling increased to 95% the amount of biomass with high settling velocities (>1.6 m/h).
- The green microalgae *Chlorella* sp. was the dominant species (>60% abundance) overall the experimental period in the R-HRAP and C-HRAP systems. The highest recycling rate (10%) decreased the dominance of *Chlorella* sp. by increasing diatoms (7.4% on average in the R-HRAP) and *Stigeoclonium* sp. (16.8% on average, only present in the R-HRAP).
- Biomass production varied within the range of 3.3-25.8 g TSS/m^2d in the R-HRAP and 5.5-25.7 g TSS/m^2d in the C-HRAP. Thus, microalgal biomass production was not

affected by recycling.

- Average COD and N-NH_4^+ removals of 80% and 97% were achieved in both HRAPs for secondary treatment of wastewater. However, the higher biomass recovery in the R-HRAP reduced TSS effluent concentration to less than 35 mg/L, generating an effluent suitable for water discharge.

On the whole, this study demonstrated that recycling can be an effective strategy to enhance biomass harvesting (up to 94%) by selecting the most rapidly settling microalgae species without compromising biomass production while enhancing the wastewater treatment performance.

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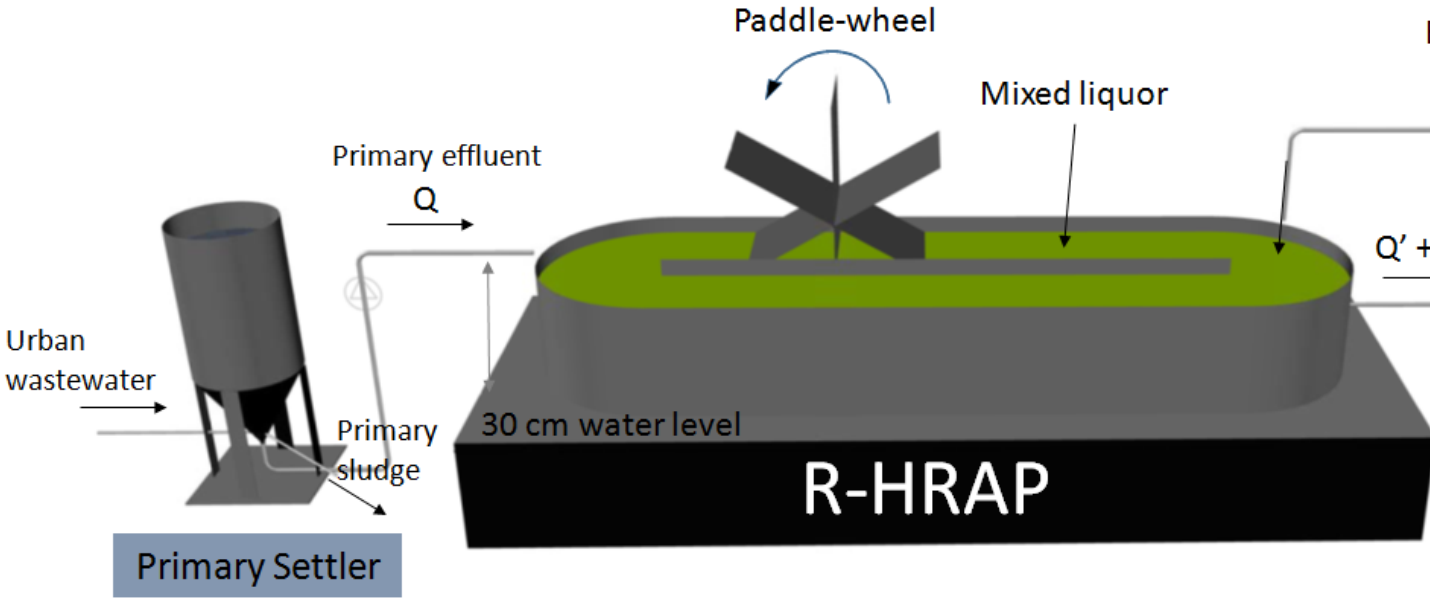

Figure 1. Schematic diagram of the process line with recycling including the primary treatment (primary settler) and the secondary treatment (high rate algal pond with biomass recycling (R-HRAP) followed by a secondary settler). Q is the primary effluent flow rate (L/d), Q' is the flow rate considering evaporation and precipitation (L/d), Q_R is the recycled flow rate (L/d) and Q_w is the harvested biomass flow rate (L/d).

Table 1. Environmental and operation parameters of the high rate algal ponds with and without biomass recycling. Average values (s.d.) of nutrient and organic matter concentration correspond to the primary effluent and biomass concentration of HRAPs mixed liquor samples taken at 12 PM. Temperature and solar radiation are average daily values (s.d.) of the period.

Parameter	**Period 1 (Mar-Apr'14) *n* = 6**	**Period 2 (May-July'14) *n* = 12**	**Period 3 (Aug-Oct'14) *n* = 8**	**Period 4 (Nov-Mar'15) *n* = 13**
Solar radiation (W/m^2)	398 (33)	446 (28)	355 (43)	234 (38)
Air temperature (ºC)	15.8 (2.1)	22.5 (3.5)	23.7 (1.8)	13.1 (2.4)
Influent flow rate (L/d)*	58.9 (3)	117.3 (5)	78.8 (5)	60.4 (6)
Influent $N\text{-}NH_4^+$ (mg/L)	30 (7)	33 (5)	36 (9)	26 (6)
Influent COD (mg/L)	381 (150)	463 (200)	318 (181)	363 (190)
HRAP HRT (days)*	8.1 (0.4)	4.2 (0.6)	6 (0.5)	7.8 (0.8)
Recycling rate (%)**	2	2	10	10
SRT (days)**	8.6 (0.5)	4.5 (0.8)	39.9 (3.5)	52.9 (2.0)
Secondary settler HRT (hours)	1.2	0.6	1	1.2
Biomass concentration in the mixed liquor (g TSS/L)**	0.36 (0.12)	0.38 (0.13)	0.46 (0.83)	0.30 (0.11)
Harvested biomass concentration (g TSS/L)**	13.3 (13.3)	20.9 (11.5)	20.2 (7.2)	11.3 (10.6)
Recycled biomass flow rate (L/d)**	0.22 (0.19)	0.20 (0.15)	0.87 (0.16)	0.76 (0.27)

*Calculated considering evaporation and precipitation rates.
** Only for the high rate algal pond with biomass recycling (R-HRAP)

549 (a)

550

551
552 (b)

553

554
555 Figure 2. Microalgal biomass concentration calculated from total suspended solids (TSS) in the
556 mixed liquor and in the effluent of secondary settlers from the high rate algal ponds with biomass
557 recycling (R-HRAP) (a) and control (C-HRAP) (b) over one year. Biomass recoveries are
558 represented by grey bars.

559

Table 2. Biomass production and biomass recovery in the high rate algal pond with biomass recycling (R-HRAP) and the control high rate algal pond (C-HRAP). Average values (s.d.) from samples taken at 12 PM.

Parameter		**Period 1 (Mar-Apr'14) *n* = 6**	**Period 2 (May-July'14) *n* = 12**	**Period 3 (Aug-Oct'14) *n* = 8**	**Period 4 (Nov-Mar'15) *n* = 13**
HRAP mixed liquor biomass concentration (mg TSS/L)	R-HRAP	359 (120)	379 (129)	459 (83)	301 (108)
	C-HRAP	273 (94)	353 (73)	197 (69)	144 (47)
Secondary settler effluent biomass concentration (mg TSS/L)	R-HRAP	23 (12)	25 (14)	30 (14)	18 (8)
	C-HRAP	54 (59)	39 (17)	34 (12)	34 (13)
Biomass recovery (%)[1]	R-HRAP	93.0 (4)	92.6 (3)	94.2 (2)	91.9 (7)
	C-HRAP	78.7 (20)	88.9 (6)	78.9 (8)	75.8 (9)
Difference between the biomass recovery from both HRAPs (%)		14 (19)	4.6 (8)	15.2 (7)	16.0 (11)
Biomass recovery with settling velocities ≥ 0.4 m/h (%)*	R-HRAP	-	98	-	92
	C-HRAP	-	93	-	36
Microalgal biomass production (g TSS/m^{2}d)[2]	R-HRAP	12.5 (3.8)	25.8 (10.7)	10.5 (4.5)	3.3 (1.7)
	C-HRAP	10.4 (3.6)	25.7 (6.9)	10.0 (3.5)	5.5 (1.8)

[1] *p*-value of 5exp-7
[2] *p*-value of 0.909
*Biomass recovery from sedimentation tests calculated as the percentage of biomass of the mixed liquor of the R-HRAP and the C-HRAP with settling velocities ≥ to 0.4 m/h.

576

577 (a)

$v \geq 6.5$ $6.5 > v \geq 1.6$ $1.6 > v \geq 0.4$ $v < 0.4$

578

579

580 (b)

581

582

583 Figure 3. Settling velocities distribution of microalgal biomass from the high rate algal pond with
584 biomass recycling (R-HRAP) (brown columns) and the control high rate algal pond (C-HRAP)
585 (green columns). Samples from period 2 (2% recycling rate) (a) and period 4 (10% recycling rate)
586 (b).

587

588

589

590 Figure 4. Microalgal biomass production in the high rate algal pond with biomass recycling (R-
591 HRAP) and the control high rate algal pond (C-HRAP).

592

593

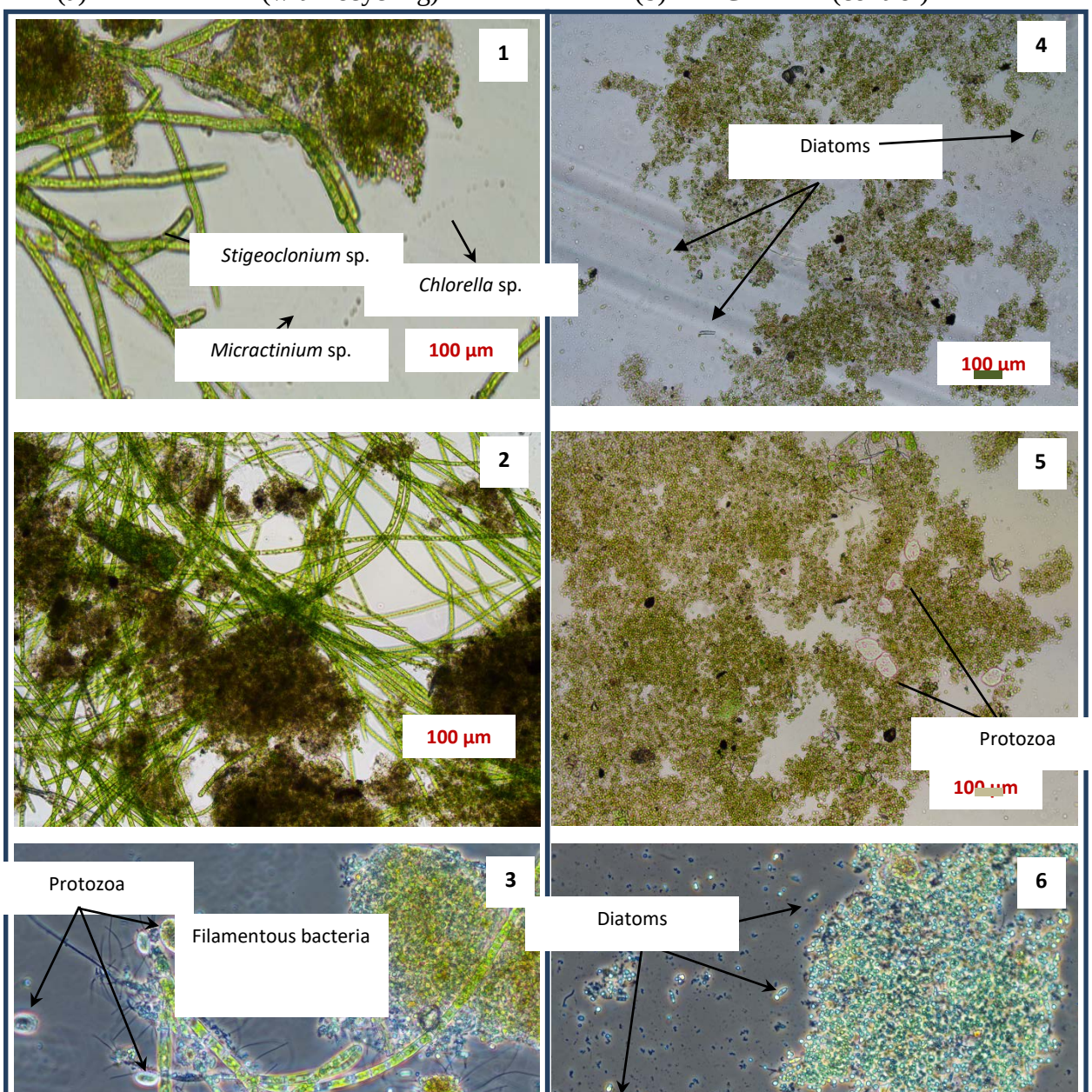
(a) R-HRAP (with recycling)
(b) C-HRAP (control)
1
Stigeoclonium sp.
Chlorella sp.
Micractinium sp.
100 µm
2
100 µm
3
Protozoa
Filamentous bacteria
4
Diatoms
100 µm
5
Protozoa
100 µm
6
Diatoms

Figure 5. Microscopic populations in the mixed liquor from samples taken in November (Period 4) from the high rate algal pond with recycling (R-HRAP) (a) (1) *Chlorella* sp. (cells immersed in flocs) and *Stigeoclonium* sp. (filamentous microalgae) and *Micractinium* sp. (2) *Chlorella* sp. (cells immersed in flocs) and *Stigeoclonium* sp. (3) *Chlorella* sp. (cells immersed in flocs), *Stigeoclonium* sp., filamentous bacteria and protozoa; and the control high rate algal pond (C-HRAP) (b) (4) *Chlorella* sp. (cells immersed in flocs) and diatoms (5) *Chlorella* sp. (cells immersed in flocs) and protozoa (6) *Chlorella* sp. flocs and some dispersed *Nitzschia* sp. and *Navicula* sp. diatoms.

(a)

(c)

(b) (d)

Figure 6. Dynamics of microalgae populations of the high rate algal pond with biomass recycling (R-HRAP) (a) (c) and the control high rate algal pond (C-HRAP) (b) (d) during 2% recycling rate (a) (b) and 10 % recycling rate period (c) (d).

690 (a)

691

692
693 (b)

694

695
696
697 Figure 7. COD (a) and N-NH_4^+ (b) removal (%) in the HRAP with biomass recycling (R-HRAP)
698 and control HRAP (C-HRAP) and primary effluent NH_4^+ (a) and COD (b) concentration (mg/L).