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Operating a semi-continuous raceway pond allows to link pH and oxygen dynamics to the interaction between microalgae and bacteria

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ABSTRACT

The impact of nutrient load and hydraulic residence time (HRT) on the dynamics of a microalgae/bacteria consortia treating different kinds of urban influents (primary settled wastewater, supernatant of digestate dewatering) was investigated. A 80 L raceway pond was operated for over 8 months. Biomass productivity was 4.1 ± 0.2 g TSS/m^2/d. While treating primary settled wastewater at 4 d HRT, nitrogen removal efficiencies were 80.1% ± 16.7% and 47.2% ± 24.8% for total Kjeldahl (TKN) and total nitrogen (TN), respectively. The relatively high TN removal suggests ammonia stripping since the pH value was close to 9.0. When increasing nutrient load with dewatering influent, the performances decreased. The HRT had to be increased to 8 d to recover a similar TKN removal efficiency (84.2% ± 9.8%) while TN removal remained negligible (1.0% ± 2.9%) due to a pH drop below 6.0. The time series decomposition technique was applied on dissolved oxygen (DO) and pH monitoring data to evaluate the dynamics of algal and bacterial processes: nitrification became the dominant process impacting pH and DO when the nutrient load increased. Algal photosynthesis decreased but slightly recovered when applying a higher HRT (8 d). Results from this study show potential for a fast and simple assessment of algal bacterial dynamics under different influencing factors.

Keywords: Wastewater treatment; Centrate; Nitrification; pH; Oxygen

1. Introduction

Since the pioneering work of Oswald and Gotaas [1], algal bacterial technology in wastewater treatment has been increasingly studied in order to develop a sustainable system for wastewater treatment as well as biomass production [2]. The core of this technology relies on synergistic interaction between microalgae and bacteria. Via photosynthesis, microalgae use light energy and inorganic carbon for their growth and metabolism, while releasing dissolved oxygen in bulk water [3]. This process promotes aerobic bacterial activity (degradation of organic matter) and increases the pH of water with a hygienization effect towards pathogens [2]. In turn, bacterial respiration provides CO_2 for microalgae, thus completing the synergistic cycle between them. With this technology, organic pollutants can be removed effectively without extensive mechanical aeration which accounts for about 50% of the total energy consumption of typical aerobic wastewater treatments [4]. Moreover, the nutrients from wastewater can be recovered by harvesting generated algal bacterial biomass, which can be used as fertilizer or as material for biofuel production [5]. It is also indicated that by inoculating activated sludge

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with microalgae in wastewater, the flocculation between microalgae and bacteria can be accelerated forming large algal bacterial flocs while keeping good treatment efficiency [6–8]. Thus, the algal bacterial biomass can be harvested efficiently by simple gravitational sedimentation [9].

Besides, as a result of early studies on photosynthesis in sewage wastewater [1], a high rate algal pond (HRAP) system was developed. A typical HRAP is an open, raceway pond with shallow water. Mixing in the pond is enabled through paddle wheeling providing vertical and horizontal dispersion and hence circulating water along the channel [2]. Due to its enhanced mixing, HRAP allows a denser algal bacterial biomass to develop, supporting a higher treatment rate than the traditional stabilization pond, thus requiring a much smaller area and retention time [10]. So far, the HRAP system has been applied to the treatment of various types of wastewater with different nutrient loads ranging from normal nutrient load such as aquaculture [9] or normal domestic wastewater [11] to high nutrient load such as centrate or piggery wastewater [12,13].

Harmonious cooperation between algae and bacteria is a key issue for successful HRAP operation. Numerous factors including nutrient loads (nitrogen and phosphorus), environmental (light, pH, or temperature) and operational (mixing, hydraulic retention time (HRT)) conditions have been identified as limiting factors of the algal bacterial cooperation and thus of the performance of the system [15]. For instance, the competition between microalgae and nitrifying biomass has been shown to vary depending on light intensity and temperature [16]. Moreover, these factors generally generate dynamic variations which require a case-to-case basis problem interpretation and process adaptation. However, data concerning the dynamics between algae and bacteria in HRAP under these effects, in particular during long-term operations is scarce.

In recent years, anaerobic digestion has become a popular solution for bioenergy production [17] and the use of its liquid effluent as a nutrient-rich source for the HRAP system promoting nutrient recovery and biomass production has gained interest [18]. Obviously, this effluent is highly loaded with nutrients, mainly ammonium nitrogen and ortho-phosphate. Depending on the upstream anaerobic digester operation, variations in hydraulic and nutrient loadings are likely to occur. Recent studies have shown the potential of microalgae/bacteria consortia in treating this sidestream effluent [19,20]. The aim of this study is to evaluate the performances of a pilot-scale HRAP under different nutrient loads and HRTs.

The HRAP was inoculated with algal bacterial biomass and operated over an 8-month period. HRT as well as nutrient loads were varied by mixing domestic wastewater and centrate from anaerobic digestion.

The performance of the system was assessed in terms of treatment efficiency, biomass production, and recovery under different conditions. In order to better assess the competition between microalgae and nitrifying bacteria, pH, and dissolved oxygen time series were statistically decomposed in order to extract the trends observed at different time-scales (photoperiod, feeding events, etc.). These data were correlated with system treatment efficiency, biomass production, and recovery.

2. Materials and methods

The pilot-scale HRAP was operated for 8 months using real influent wastewater as a nutrient source. First, primary settled wastewater was used to represent a low nutrient loading rate. After 135 d of operation, the primary settled wastewater was mixed with centrate from anaerobic digestion in order to increase the nutrient load. The influence of HRT was then studied by increasing it from 4 to 8 d at day 206 of operation. Pilot HRAP operation data including influent and effluent wastewater, biomass, and physiochemical characteristics were collected over the course of all the operational period.

2.1. Pilot HRAP operation

2.1.1. Wastewater and algal bacterial inoculums

Primary treated and centrate wastewaters were collected from Strasbourg La Wantzenau (67000, France) wastewater treatment plant (WWTP). This plant has a capacity of 1,000,000 people equivalents (PE). Its water line consists of mechanical pretreatments, primary settling, activated sludge process with extended aeration (performing nitrification and denitrification). The sludge line consists of sludge mechanical thickening, two mesophilic anaerobic digesters, and post-centrifugation.

Influents were sampled every 1 or 2 weeks and stored in a cooling tank (CV 420, JAPY, France) at 4°C. Centrate wastewater coming from an anaerobic digestion reactor was added to determine the impact of high nutrient load on HRAP performance and algal bacterial dynamics. In case of centrate wastewater collection, floating solids such as sludge and other materials were discarded before mixing with primary treated wastewater in the storage tank. Influent concentrations are presented in Table 1. The first period of operation (LN_4d) corresponds to reactor feeding with primary settled wastewater with relatively low concentrations of TKN and TP. During the two following periods (HN_4d and HN_8d), the reactor was fed with a mixture of primary settled and centrate wastewater: the COD concentration increased to some extent but TKN and TP concentrations increased much more.

The HRAP was inoculated with algal bacterial (Al-Bac) biomass including a mixture of microalgae and activated sludge (1:1 TSS ratio). These inoculums were collected from a local WWTP (Rosheim, France) to enhance cooperation and improve biofloculation between algae and bacteria [8]. The biomass was then cultured in batch reactors prior the HRAP inoculation [8,9]. No additional inoculation was performed afterwards during the entire pilot operation. Microscopic observation (light microscope Olympus BH-2) showed that the mixture of microalgae predominantly contained *Chlorella* sp., *Ulothrix* sp., *Scenedesmus* sp., *Pseudanabaenaceae* sp., and *Nitzschia* sp. which are commonly found in wastewater [21].

2.1.2. Pilot description

The pilot HRAP consists of a single loop raceway pond with two straight channels separated by a wall and connected by a 180° bend at each end. The total wet area is

Table 1
Influent concentrations for all stages

Parameters	LN_4d[a]	HN_4d[a]	HN_8d[a]
COD (mgO_2/L)	286.1 ± 102.4	339.9 ± 207.5	430.2 ± 243.8
TKN (mgN/L)	34.8 ± 16.1	123.5 ± 33.8	114.5 ± 24.4
NO_3 (mgN/L)	0.2 ± 0.3	0.2 ± 0.2	0.3 ± 0.3
NO_2 (mgN/L)	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.3
NO_2 (mgN/L)	2.1 ± 5.1	0.4 ± 0.3	0.4 ± 0.4
TP (mgP/L)	4.3 ± 1.3	31.4 ± 11.5	41.2 ± 14.3

[a]LN_4d: low nutrients load and 4 d HRT; HN_4d: high nutrients load and 4 d HRT; HN_8d: high nutrients load and 8 d HRT.

0.72 m^2. The pond has a high length-to-width ratio (*L*/*W*) of 19, which is in the optimal range suggested by Hadiyanto et al. [22] for improving hydraulic efficiency. A deflector was also placed at each end of the channel to homogenize the flow and decrease shear stress and dead zones [22,23]. Liquid circulation in the pilot was ensured by a six-blades 0.75m diameter paddlewheel driven by a brushed DC motor (DMN37K, 24V, Nidec Servo Corporation, Japan) which was controlled by a bench power supply (ISO-TECH IPS303DD, England). The pilot and paddlewheel were made of transparent plastic (Fig. 1).

Following the raceway reactor, the biomass was clarified by a gravity settler. The settler was made of transparent plastic. Its wet surface was 0.055 m^2 and the total volume was 20 L. The height of the end wall determined the water level in the HRAP. The inlet position was located near the bottom for better sedimentation [24] (Fig. 1). To minimize the impact of floating sludge due to denitrification in the settler that may negatively impact the effluent quality [25], a baffle was positioned right next to the end wall. Settled biomass was harvested via a pipe connected to a peristaltic pump (Masterflex L/S Economy).

2.1.3. Operational conditions

The pilot HRAP was operated indoors during 246 d from August 2017 to April 2018 (Table 2). The operating conditions were varied to investigate the impact of different nutrient loads and HRTs on the growth of Al-Bac biomass. The experimental setup is illustrated in Fig. 2.

In all the experiments, the water level was maintained at 0.11 m, giving 80 L of total volume. The rotating speed of the paddle wheel was maintained at 11.6 rpm for better mixing and mass transfer, giving the mid-channel an average

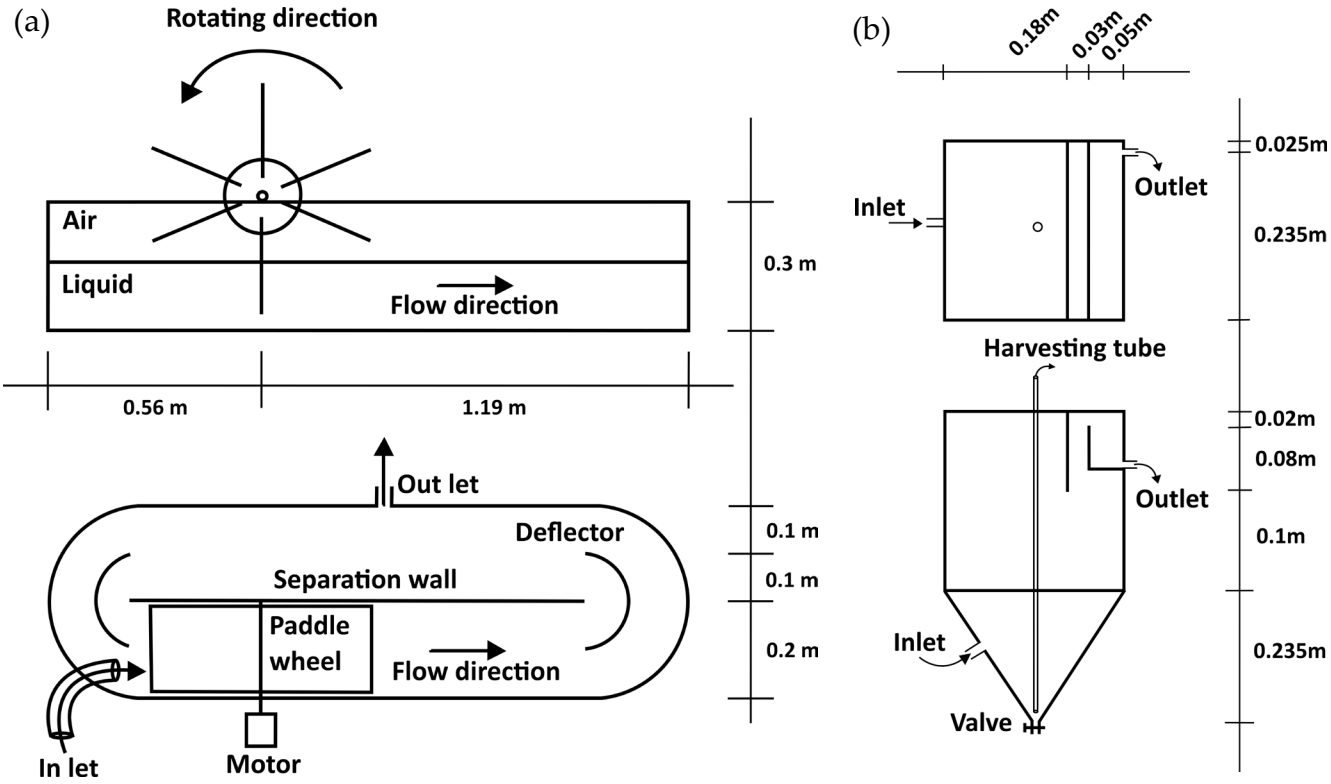

Fig. 1. Side view and top view of (a) the pilot raceway pond and (b) settler.

Table 2
Operational characteristics of different stages

Stage	Name [a]	Time	Feed wastewater[b]	HRT (d)	Study objective
1	LN_4d	August–December 2017 (135 d)	P	4	Long term performance
2	HN_4d	December 2017–February 2018 (53 d)	P + C (2v:1v)	4	High nutrient impact
3	HN_8d	February–April 2018 (57d)	P + C (2v:1v)	8	High HRT impact

[a]LN_4d: low nutrients load and 4 d HRT; HN_4d: high nutrients load and 4 d HRT; HN_8d: high nutrients load and 8 d HRT.
[b]P/C is primary treated/centrate wastewater and v is volume.

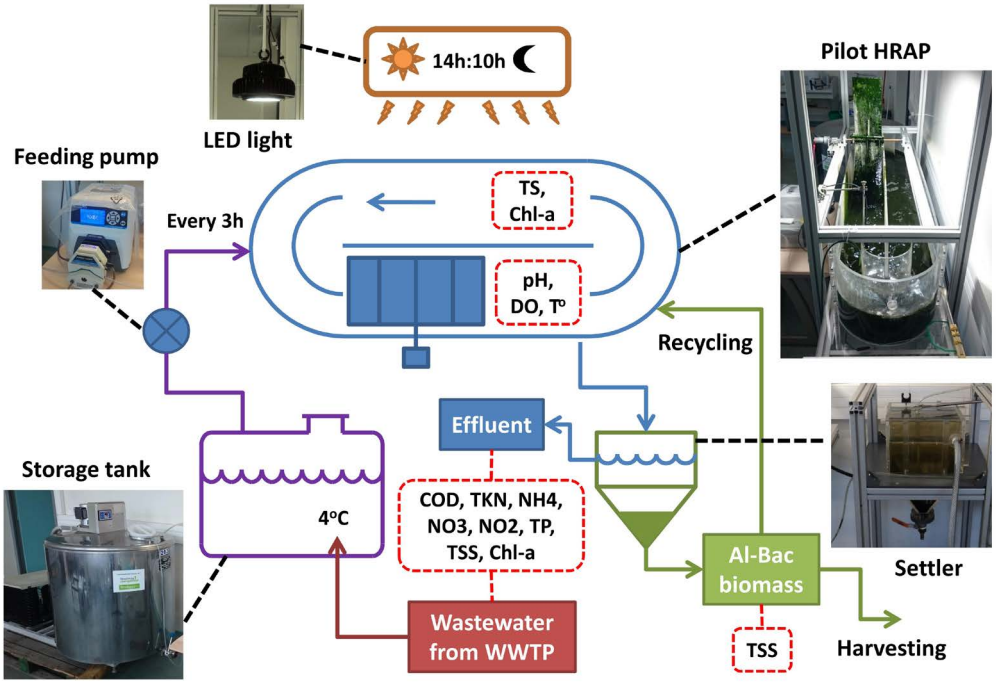

Fig. 2. General illustration of HRAP experimental setup.

velocity of 0.44 m/s [26]. Illumination was provided by a high-power LED light (ARIAH2 HIGHBAY, ENLITE, UK) positioned on top of the pilot at a vertical distance of the water surface of 0.8 m, providing a constant light intensity of 210 μE/s/m^2 at the water surface. The light intensity applied was in the optimal range enhancing algal growth [4] while the use of LED light was suggested by [27]. A timer was connected to the light source to obtain a photoperiod of 14 h light/10 h dark. It was chosen to favor the growth of algae but taking into consideration practical perspective [28].

In order to visualize pH and dissolved oxygen (DO) dynamics due to algal/bacterial interactions, the HRAP was fed discontinuously. Feeding events occurred every 3 h, owing to a peristaltic pump (Masterflex L/S Standard Digital Pump System). By adjusting the pumping rate, the desired HRT was achieved. From day 30 of the first stage (Table 2), except for the hour when feeding occurred, a volume of 0.5 L from the bottom of the settler was recycled to the HRAP every hour. The recycling was performed by a peristaltic pump (Masterflex L/S Standard Digital Pump System, USA) controlled by a timer. This recycling strategy was implemented in order to increase removal efficiency by bioflocculation/aggregation of the algal/bacterial aggregates in a similar way to sludge recirculation in the activated sludge process [29]. Biomass harvesting (wastage from the system) was performed two times per week: all thickened biomass at the bottom of the settler was then harvested by peristaltic pumping.

2.1.4. Sample collection and analytical procedures

Dissolved Oxygen (DO) (Portavo 907 Multi Oxy Knick), pH, and temperature (WTW pocket pH meter kits pH330) were measured every 5–10 min at the central point of the channel located downstream the paddle wheel. The probes were positioned 45° along the flow to avoid biomass clogging.

Influent and effluent were sampled once per week for the analysis of chemical oxygen demand (COD) (Nanocolor® COD 1500 according to [30]), total Kjeldahl nitrogen (TKN–N) [31], ammonium nitrogen (NH_4–N) (NF EN ISO 14911), nitrate–nitrogen (NO_3–N) [32], nitrite–nitrogen (NO_2–N) [32], and total phosphorus (TP) (Nanocolor® ortho- and total

phosphate 15 according to DIN EN ISO 6878-D11) [33]. Total nitrogen (TN) was calculated as the sum of total Kjeldahl nitrogen, ammonium nitrogen, nitrate–nitrogen, and nitrite–nitrogen. Total suspended solids (TSS) [34] and chlorophyll a (Chl-a) [35] were analyzed in samples collected from the HRAP at the same frequency while chlorophyll-a in inlet wastewater was only measured every 2 weeks.

2.2. Data analysis

2.2.1. System performance analysis

The productivity (Eq. (1)) and solids retention time (SRT) (Eq. (2)) of Al-Bac biomass were calculated using simple mass balance equations [36]:

$$P = V\frac{dX_{\text{Al-Bac}}}{dt} - Q_{\text{in}} \times X_{\text{in}} + Q_{\text{out}} \times X_{\text{out}} + Q_{\text{harvested}} \times X_{\text{harvested}} \quad (1)$$

$$\text{SRT} = \frac{X_{\text{Al-Bac}} \times V}{Q_{\text{harvested}} \times X_{\text{harvested}} + Q_{\text{out}} \times X_{\text{out}}} \quad (2)$$

where P is the productivity in mg TSS/d, $X_{\text{Al-Bac}}/X_{\text{in}}/X_{\text{out}}/X_{\text{wastage}}$ are the concentrations of suspended solids (mg/L) in HRAP, inlet wastewater, treated effluent, and harvested biomass respectively. $Q_{\text{in}}/Q_{\text{out}}/Q_{\text{wastage}}$ are the influent/effluent/harvesting flow rates in L/d. V is the total volume of HRAP in L and SRT is the solids retention time of Al-Bac biomass in days. In SRT calculation (Eq. (2)), the influent solids were neglected [36].

For example, during the first week of operation, Al-Bac biomass concentration increased from 740 to 1,003 mg/L within 7 d. The inlet and outlet flowrates were both 20 L/d. Inlet and outlet TSS concentrations were 41 and 10 mg/L, respectively. No harvesting was carried out during this period. The numerical application of Eq. (1) yields:

$$P = 80 \times \frac{1{,}003 - 740}{7} - 20 \times 41 + 20 \times 10 = 2{,}385 \text{ mg TSS/d} \quad (3)$$

Treatment efficiencies were calculated daily as follows:

$$E = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \times 100\% \quad (4)$$

where E is the treatment efficiency, C_{in} and C_{out} are the concentrations at the influent and effluent, respectively.

2.2.2. Algal bacterial dynamics analysis

The factors influencing DO dynamics in the HRAP were evaluated by analyzing the recorded DO profile. The observed DO dynamics in the HRAP with (Eq. (5)) and without light (Eq. (6)) can be generally described as:

$$\frac{dO_{2,\text{light}}}{dt} = \text{OTR} - \text{OUR}_{\text{light}} + \text{OPR} \quad (5)$$

$$\frac{dO_{2,\text{dark}}}{dt} = \text{OTR} - \text{OUR}_{\text{dark}} \quad (6)$$

where oxygen transfer rate (OTR), oxygen uptake rates (OUR), and oxygen production rates (OPR) stand for oxygen transfer, uptake, and production rates in light and dark conditions expressed in mg/L/d, respectively.

2.2.3. Statistical analysis

Data statistical analysis was performed using R software (version 3.3.1, 2016–06–21). The difference between data obtained from three operational stages was determined to evaluate the impact of the different conditions.

The comparison of two data sets started with normal distribution determination using the Shapiro–Wilk test and then the homoscedasticity evaluation by Fisher–Snedecor test. In case of normal distribution, either the Student *t*-test or the Welch test was applied for equal or unequal variances, respectively. Otherwise, the Mann–Whitney–Wilcoxon test was used. For multiple dataset comparison, normally distributed datasets were determined for homoscedasticity by the Bartlett test. Then significant differences were analyzed using the ANOVA or ANOVA-Welch correction for equal or unequal variances, respectively, followed by pairwise *t*-test. Otherwise, the Kruskal–Wallis test followed by Pairwise Wilcoxon Rank Sum Tests was used. All tests were applied with the threshold value of 0.05. Mean values were presented with standard deviations.

Moreover, to separate the impact of feeding and light/dark cycle, time series decomposition was performed using the seasonal and trend decomposition using loess (STL) (stl function in R) [37]. The time series decomposition allowed to split time series data of DO and pH into three components series including seasonal, trend, and random (or noise). Seasonal data is a periodic pattern while trend data is the underlying trend of the series over a long period. Random or noise data (remainder) is the residual of the original time series after seasonal and trend data are removed. As the feeding and light/dark cycle occurred repeatedly during the entire operation, the decomposition technique applied in this study followed the additive model (time series = seasonal + trend + remainder). In order to perform decomposition, it is important to determine the size of the seasonality. In this study, different frequencies were applied depending on the recording intervals of the time series data.

3. Results and discussion

3.1. Treatment performance

The wastewater treatment performance was monitored by following the organic pollution (as COD) and nutrient (nitrogen, phosphorus) removal efficiencies. As expected, centrate wastewater injection during the last two stages (HN_4d and HN_8d) provided higher influent nutrient concentrations (TKN, TP) ($p < 0.05$) compared to the first stage (LN_4d) (Table 1). The influent C:N ratio (calculated following [38]) decreased from 3.7 ± 1.9 to 1.0 ± 0.5 and 1.5 ± 0.6 for stages LN_4d, HN_4d, and HN_8d, respectively. This result was in agreement with the indication that ammonium nitrogen and phosphorus are the main constituents in liquid effluent from the anaerobic digestion system (centrate wastewater) [19].

Removal rates and efficiencies in different stages are presented in Table 3. Throughout the experiment, COD and TKN removal efficiencies were at high level in comparison with other studies applying algal bacterial biomass [9,12,39–41]. The high COD removal efficiency was in accordance with high level of DO in the reactor in all the stages (Table 4): indeed, DO is required by heterotrophic bacteria to oxidize organic matter efficiently [4].

Fig. 3 displays concentrations of Kjeldahl (TKN) nitrogen in the influent as well as the different nitrogen species in the effluent of the HRAP. It is clear from this Fig. that the fate of N in the raceway reactor was mainly impacted by nitrification starting from day 50. Except for a short NO_2–N peak between days 10 and 31, nitrification was complete as no significant NO_2–N concentration was detected afterwards. However, during the first stage of reactor operation (LN_4d), a high loss of nitrogen occurred (total nitrogen removal yield of 47.2% ± 24.8%). This is comparable with other long term HRAP studies (Table 3). As discussed in Section 3.3.2 (Algal bacterial dynamics under different nutrient loads and HRTs), pond environment was aerobic, and denitrification was not likely to occur. Given the order of magnitude of this loss and biomass productivity, the accumulation inside biomass cells cannot explain this behavior. Finally, ammonia (NH_3) volatilization most probably explains this nitrogen loss: indeed, the average pH in the pond during this period was 8.4 (Table 4) and it was often above 9.2 (Fig. 6). This is close to the NH_4^+/NH_3 acid-base dissociation constant (pKa = 9.25). This was confirmed during the second and third phases with high nutrient load: pH was around 6.0 (Table 4) and the loss of nitrogen became insignificant.

Phosphorus can be removed by assimilation in algal or bacterial cells. This accounts for a small proportion of algal dry weight [3]. Moreover, it was indicated that the atomic ratio between nitrogen and phosphorus (N/P) should range from 10 to 30. A value outside this range is an indication for P or N limitation [16]. In this study, the average atomic N/P ratios observed in influent wastewater were 18.6 ± 7.2, 9.2 ± 1.6, and 6.7 ± 2.5 for stages LN_4d, HN_4d, and HN_8d, respectively. With the favorable N/P ratio and a low level

Table 3
Removal rates and removal efficiencies of different wastewater characteristics for all stages

Parameters		LN_4d[a]	HN_4d[a]	HN_8d[a]	Literature[b]
COD removal	Rate (mgO_2/L/d)	56.4 ± 25.8	49.2 ± 39.7	42.8 ± 29.0	15–93
	Efficiency (%)	77.0 ± 11.4	49.0 ± 25.2	75.0 ± 9.7	
TKN removal	Rate (mgN/L/d)	8.5 ± 4.1	24.1 ± 6.7	12.3 ± 2.5	35–94
	Efficiency (%)	80.1 ± 16.7	78.9 ± 9.3	84.2 ± 9.8	
TN removal	Rate (mgN/L/d)	4.7 ± 3.5	3.9 ± 6.1	0.2 ± 0.5	20–88
	Efficiency (%)	47.2 ± 24.8	12.4 ± 16.0	1.0 ± 2.9	
TP removal	Rate (mgP/L/d)	0.6 ± 0.3	1.2 ± 1.3	0.2 ± 0.3	10–94
	Efficiency (%)	57.7 ± 18.7	13.9 ± 13.6	4.7 ± 7.9	

[a]LN_4d: low nutrients load and 4 d HRT; HN_4d: high nutrients load and 4 d HRT; HN_8d: high nutrients load and 8 d HRT.
[b]Updated data (since 2003) of high rate algal pond operations from [9,12,39–41]. Data of COD removal and TKN removal also included BOD_5 and ammonium nitrogen removal efficiencies, respectively.

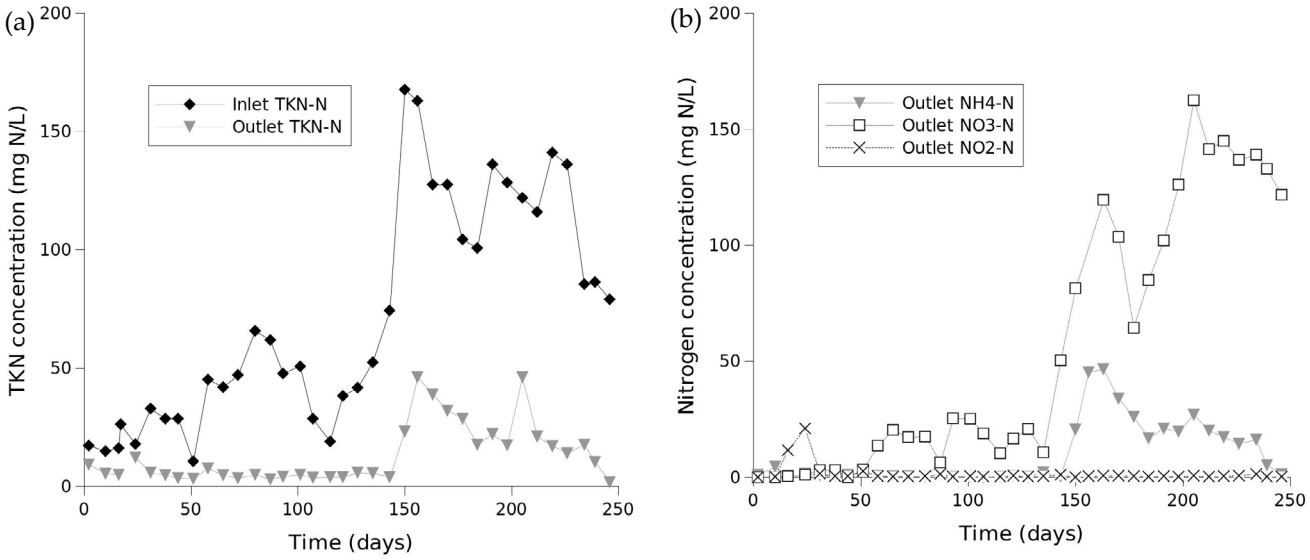

Fig. 3. Nitrogen evolution in time (a) TKN concentrations and (b) outlet N concentrations.

of influent TP in the first stage (Table 1), high TP removal efficiency of 57.7% was obtained compared to annual TP removal of 32% reported by García et al. [42]. The high pH observed in this phase could also explain this phenomenon since precipitation becomes significant under these conditions. The very low TP removal efficiency observed in the last stages (HN_4d and HN_8d) where pH was much lower also supports this assumption. Moreover, during the last two stages, influent N/P ratios were low, thus suggesting N limitation condition [16] which may contribute to the low TP removal efficiency.

3.2. Biomass production and recovery

Biomass productivity and recovery (harvesting efficiency) were assessed by performing the suspended solids mass balance over the entire system. Total algal bacterial (Al-Bac) biomass level increased continuously from the first until the last stage ($p < 0.05$, Fig. 4). As light and temperature conditions were stable throughout the experiment (Table 4), this result suggests the positive impact of high nutrient load on Al-Bac biomass growth. When the loading rate in stage HN_8d was decreased to obtain higher HRT, the SRT increased and the biomass level increased in the reactor (Table 4). As similar harvesting and recycling frequencies were maintained during the entire operation, lower biomass flowing out of the reactor resulted in lower harvesting rate. This caused extremely high Al-Bac biomass concentrations obtained in the last stage compared to the previous stages (Fig. 4) as well as biomass levels from 0.1 to 0.75 g/L commonly reported for large scale HRAP systems [9,29,43].

Concerning algae specifically, adding digestion centrate showed minor impact in algal growth as Chl-a level was relatively stable throughout the first two stages LN_4d and HN_4d ($p > 0.05$) (Table 4). During the last stage (HN_8d), a higher Chl-a level would be expected. Surprisingly, a decrease in Chl-a level was observed, reaching a similar level with previous stages ($p > 0.05$, Fig. 4). Hence, Chl-a/Al-Bac biomass ratio decreased from 2.6 ± 1.5 to 1.2 ± 0.5 and 0.8 ± 0.6 mg Chl-a/g TSS for the stages LN_4d, HN_4d, and HN_8d, respectively. Due to the high TSS concentration,

Table 4
Physio-chemical and biomass monitoring parameters for different stages

Stages	DO (mgO_2/L)	pH	Temperature (°C)	Al-Bac (gTSS/L)	Chl-a (mg/L)	Harvest rate (g/d)	SRT (d)	Recovery (%)
LN_4d	8.0 ± 2.4	8.4 ± 0.6	18.2 ± 2.5	1.6 ± 0.5	3.6 ± 1.6	3.8 ± 2.0[a]	40.9 ± 24.8[a]	99.1 ± 0.7
HN_4d	7.3 ± 1.8	6.7 ± 1.0	14.8 ± 1.7	2.5 ± 0.3	3.0 ± 1.1	4.3 ± 1.8	48.8 ± 19.5	98.9 ± 0.4
HN_8d	7.4 ± 1.9	5.2 ± 0.5	15.6 ± 1.4	4.0 ± 0.4	3.4 ± 2.8	2.1 ± 2.3	175.7 ± 91.3	99.6 ± 0.2

[a]from day 16 until 135.

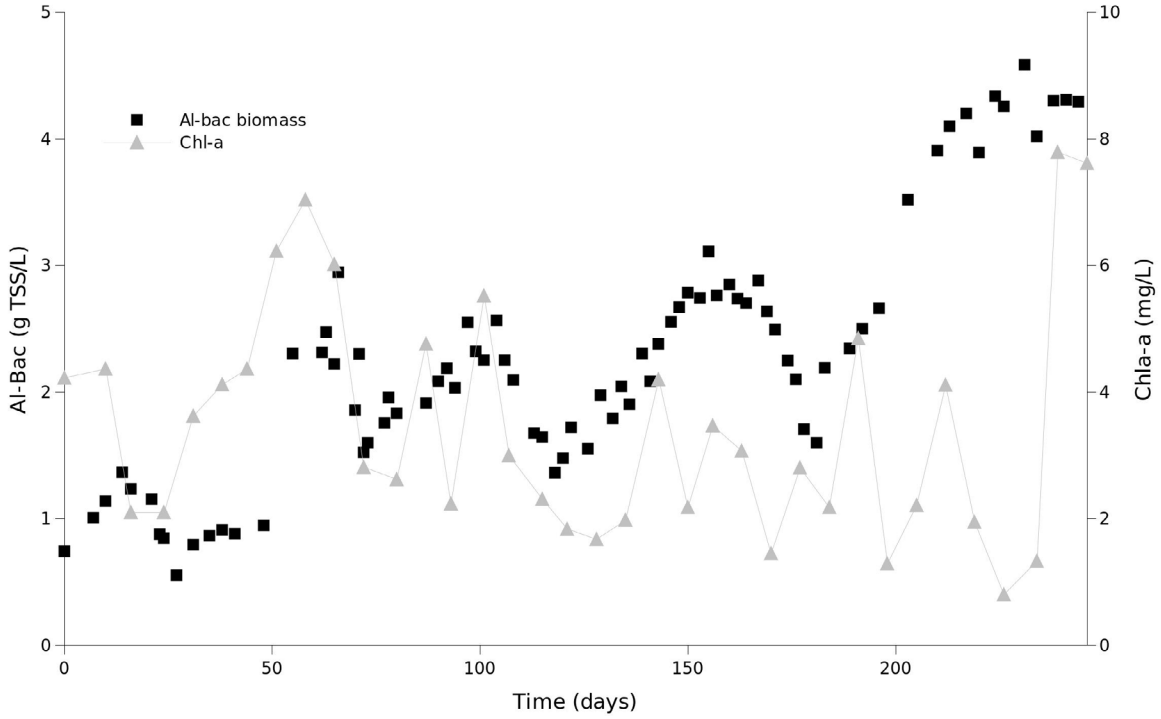

Fig. 4. Al-Bac biomass and Chl-a evolution in time.

the shading effect probably explains this decrease as well as the negative impact on algal growth [44].

This behavior was confirmed by the pH decrease observed during the last two stages ($p < 0.05$) which was mainly due to the increase of autotrophic bacterial activity (nitrification), in particular during the last stage (Table 4). Yet the decrease in DO level ($p < 0.05$) was not significant. The small decrease in DO may be due to the high degree of mixing resulting in a high air-liquid oxygen mass transfer rate in the reactor [26] that extensively provided DO supporting photosynthetic aeration. Moreover, as a high Al-Bac biomass level was maintained in the reactor, in particular during the last stage, light penetration was limited, leading to a reduction in algal productivity [16].

Average Al-Bac biomass productivity was 36.9 ± 1.8 g TSS/m^3/d. This is comparable with other HRAP treatment systems using algal bacterial biomass (from 4 to 47 g/m^3/d) [9]. The average Chl-a productivity was 113.3 ± 5.2 mg Chl-a/m^3/d during the operation resulting in a rough algal productivity of 7.6 g/m^3/d. These values were at a lower level compared to other HRAP systems using algae and wastewater born bacteria for treatment which achieved algal bacterial biomass productivity of 62.8–82.3 g/m^3/d and algal productivity of 35.8–66.4 g/m^3/d [29]. However, Chl-a content is an indirect way to estimate algal biomass. Indeed, its content in algal cell varies depending on factors such as species and culturing conditions (wastewater composition, etc.) as well as algal bacterial interactions. Despite the low algal productivity, biomass recovery was more than 99% by simple gravity settling for the entire period the operational conditions applied. The result was similar to an earlier study on the application of HRAP inoculated with algae and activated sludge for wastewater treatment [45]. The interest of this technique to improve algal biomass settleability is reconfirmed. However, more studies should be performed to improve Al-Bac biomass and algal production.

3.3. Impact of different nutrient loads and HRTs on algal bacterial dynamics

The results presented above show that the nitrification process is a major mechanism impacting nutrient removal efficiency and possibly algal productivity. Further investigation on the impact of variation in nutrient load and HRT on the dynamic between algae and bacteria in the HRAP system was conducted. Due to both discontinuous feedings of the pilot HRAP and photoperiod, variations of both pH and DO occurred at two timescales: hourly basis following batch feeding and daily basis following light/dark cycles and photosynthetic activity. The analysis of these dynamic variations potentially allows further insight into algal and bacterial biomass activities and interactions. By studying the variation of DO and pH measured in time series in response to environmental changes, knowledge of the algal bacterial kinetic processes can be deduced [46]. Owing to the well-controlled condition of the indoor environment, constant patterns in DO and pH profiles were obtained during all stages. The variation in these patterns can be related to the changes in operational conditions.

3.3.1. Analysis of DO and pH time series

In the HRAP system, the oxygen level is mainly influenced by illumination. With exposure to light, equilibrium between algal photosynthetic production and microbial respiration is the main factor affecting DO concentration within the reactor. This usually leads to a high value of DO, in particular when strong illumination occurs [2]. Without illumination, algal photosynthesis is stopped, leaving microbial respiration as the dominant process resulting in low level of oxygen in the reactor at night. In addition, optimal mixing was applied in this study resulting in a high level of air-liquid oxygen transfer in the HRAP [26]. Its influence on the DO level required to consider the phenomena of oxygen stripping, as over saturation conditions can occur during the day. When DO level is lower than saturation value, oxygen is transferred in the liquid at a high rate [47].

Besides illumination, the feeding of fresh wastewater also impacts the DO level in the reactor [8]. Immediately after batch feeding event, the DO level in the HRAP quickly decreased due to acceleration in bacterial oxidation processes (heterotrophic organic carbon oxidation and nitrification). Then, the DO level increased again at a lower rate that could be attributed to photosynthetic aeration and/or gas–liquid mass transfer.

The pH profile displayed a similar pattern. During the day, the dominant process is algal photosynthesis, causing a pH increase while nitrification or microbial respiration results in pH reduction at night [16]. Over the course of one batch, the addition of fresh influent is also followed by a sharp pH decrease due to nitrification before a gradual increase until the next feeding [8].

Throughout the experiment, only one pattern was observed in the DO profile (top part of Fig. 5) while the pH pattern (top part of Figs. 6a and b) completely shifted after high nutrient load wastewater was introduced. Two types of variations occurred within the DO and pH profiles. The large magnitude variation occurring daily is attributed to the response of the system to diurnal change in light which is commonly the case in HRAP systems [9]. The small magnitude variation occurring with greater frequency is in accordance with the feeding pattern of semi-continuous operation in which new wastewater was fed to the HRAP every 3 h. Similar fluctuation was reported by Su et al. [8] when cultivating algal bacterial biomass with wastewater in batch reactor.

By studying these variations in different stages, the impact of nutrient load and HRT on algal bacterial processes in the HRAP could be determined. However, these oscillations occurred simultaneously, hampering the identification of HRT and nutrient load impact on DO and pH fluctuations. Time decomposition technique [37] is widely applied to decompose the time series data into different components [48]. It was therefore used in this study to separate the diurnal variations of pH and DO from those induced by the feeding phases.

According to this method, the obtained time series data can be considered as the sum of different components series which vary differently through time namely seasonal, trend, and remainder (Fig. 5) [37]. In this study, the variations due to feeding were repeated every 3 h (0.125 d) for the entire

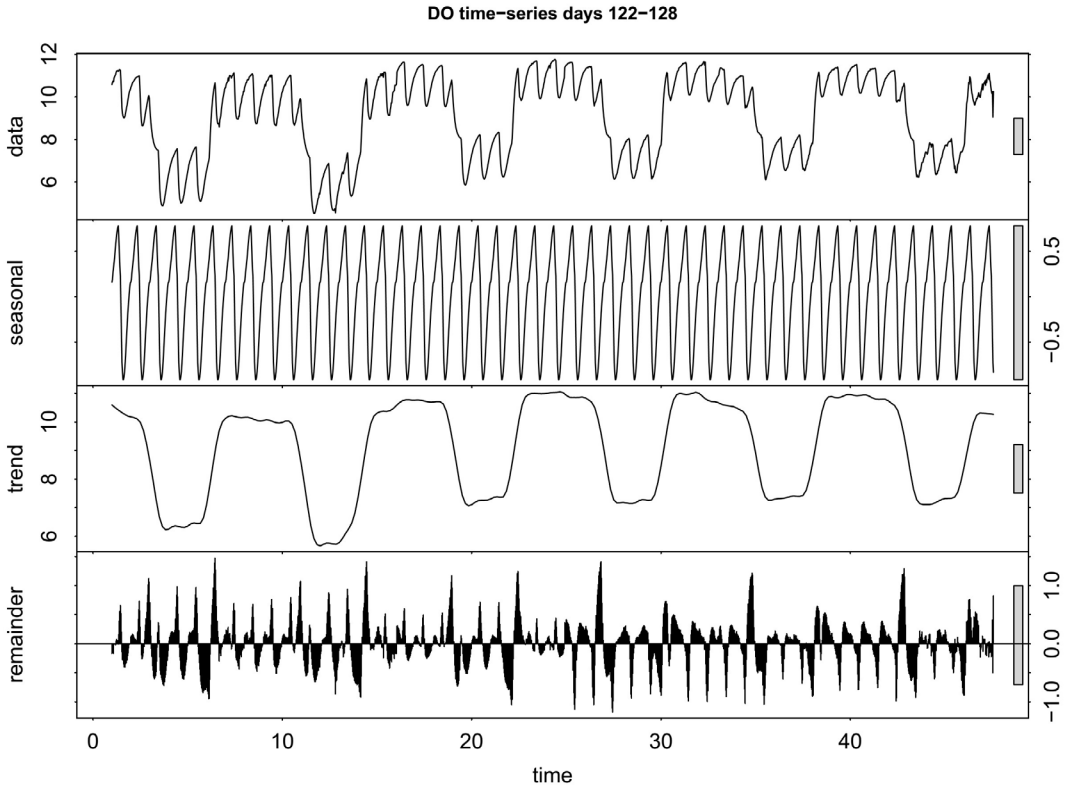

Fig. 5. Example of decomposition of time series DO data (time scale in 0.125 d and DO data in mg/L).

day and this pattern was considered as "seasonal variation." Besides, in comparison with the time scale of feeding, variations due to light and dark cycles occurred with a significantly longer period, hence corresponding to the trend component. The last "remainder" component was the residual data (noise) after seasonal and trend series were removed [48]. Examples of DO and pH decomposed time series data are presented in Figs. 5 and 6. Decomposed seasonal data represents DO and pH variation due to feeding while the trend data is attributed to their diurnal variation.

3.3.2. Algal bacterial dynamics under different nutrient loads and HRTs

The DO trend data represents the difference between DO levels measured during day and night (Fig. 5). According to Eqs. (4) and (5) and knowing the OTR from a previous study [26], the OPR and OUR values for different periods could be derived (Fig. 7). It is important to notice that, although night time algal respiration should be considered separately [49], constant algal respiration rate during day and night was commonly applied in algal bacterial kinetic model [50]. Hence, in this study, OUR was assumed to be constant during day and night. In the first stage (LN_4d), the average OPR and OUR were at the same level ($p > 0.05$) with values of 210.0 ± 25.5 and 222.5 ± 53.0 mgO_2/L/d, respectively. After addition of centrate wastewater, the average OPR decreased to 169.2 ± 13.8 mgO_2/L/d in stage HN_4d ($p < 0.05$) and then increased slightly to 191.9 ± 30.8 mgO_2/L/d in the last stage ($p > 0.05$). At the same time, average OUR values increased to 333.9 ± 62.1 mgO_2/L/d in stage HN_4d and 318.6 ± 78.9 mgO_2/L/d in stage (HN_8d) which were higher than OPR values in the same periods ($p < 0.05$).

Higher OUR levels obtained in the latter stages (HN_4d and HN_8d) indicate an increase of bacterial activity in the HRAP reactor. This result confirms that nitrifying bacteria growth increased significantly following the addition of digestion centrate: as stable COD removal rate was obtained for the entire operation (Table 3), similar heterotrophic bacterial activities in all stages were expected while the improvement of TKN removal rate indicates an enhancement in nitrifying bacterial activity after adding centrate wastewater. Hence, the OUR increase is mainly related to nitrification enhancement. Considering OPR, although a stable level of Chl-a and thus algal biomass was observed during all stages (Table 4), a lower OPR level in the second stage (HN_4d) indicates a decrease in algal photosynthesis. However, in the last stage (HN_8d), an OPR level in HRAP similar to the first stage suggests a slight increase in algal photosynthesis although the impact on DO level was negligible due to high nitrification rate.

The variance of trend and seasonal data for DO and pH was also analyzed (Table 5). The results obtained for OPR and OUR calculation were in agreement with the variation in DO trend data, which represents the gap between DO levels during day and night. Significant lower variance of DO trend data belonged to the stage HN_4d while higher values were obtained in the other stages (LN_4d and HN_8d).

Considering DO variation due to feeding (seasonal data), constant variance was obtained during day and night

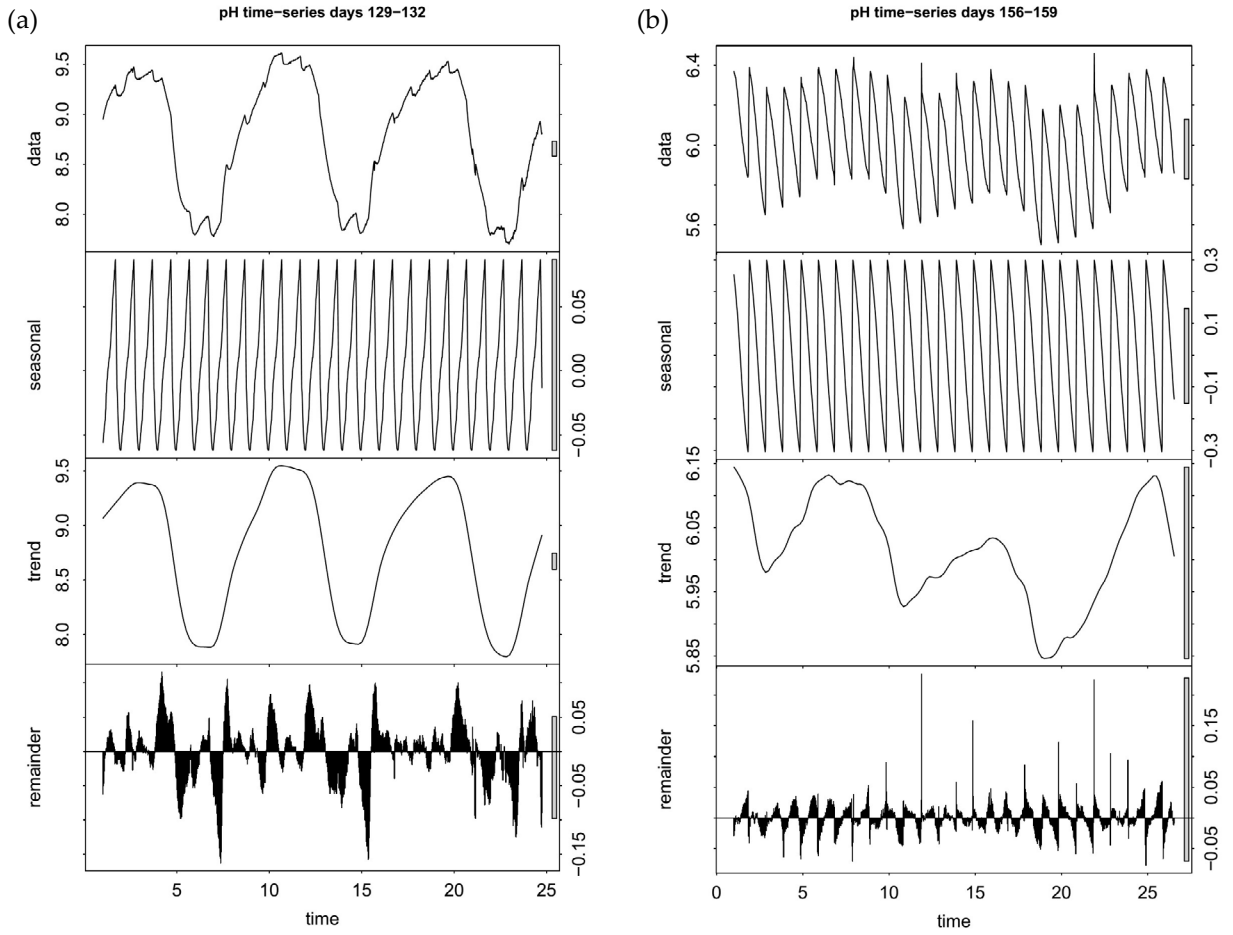

Fig. 6. Time decomposition of time series pH data recorded two weeks before (a) and after (b) feeding wastewater with high nutrient load in the HRAP (time scale in 0.125 d).

(Fig. 5). This suggests that OTR and OUR were the major factors governing DO variation due to feeding. Significant reduction in DO seasonal data in stages HN_4d and HN_8d can be attributed to the increase in nitrification which also increases oxygen uptake rate.

Significant changes ($p < 0.05$) were noticed in both trend and seasonal variation of pH data for all stages (Table 5). High algal photosynthesis with low nitrification during the first stage resulted in a high pH variation between day and night and low variation due to feeding (Table 5). However, the higher pH variability due to feeding and lower day-night pH variation confirms the dominance of nitrification after high nutrient load was introduced (stages HN_4d and HN_8d). It should be noticed that the decrease in pH (from values around 8.5 to around 6.0) due to nitrification at the beginning of the stage HN_4d was far exceeding the other factors. Therefore, the data shown in Table 5 include the variances calculated for the HN_4d stage without considering this transition period. This stationary period was therefore observed when comparing variances of trend and seasonal pH data in the entire second stage without considering transition.

Table 5
Variances of decomposed DO (mg/L) and pH data for different stages

Variances	LN_4d	HN_4d	HN_4d stationary[a]	HN_8d
DO_Trend	3.40	2.55	2.17	3.18
DO_Seasonal	0.33	0.28	0.17	0.19
pH_Trend	0.368	0.817	0.012	0.068
pH_Seasonal	0.003	0.021	0.043	0.062

[a]data without transitional period due to microbial adaptation with new wastewater.

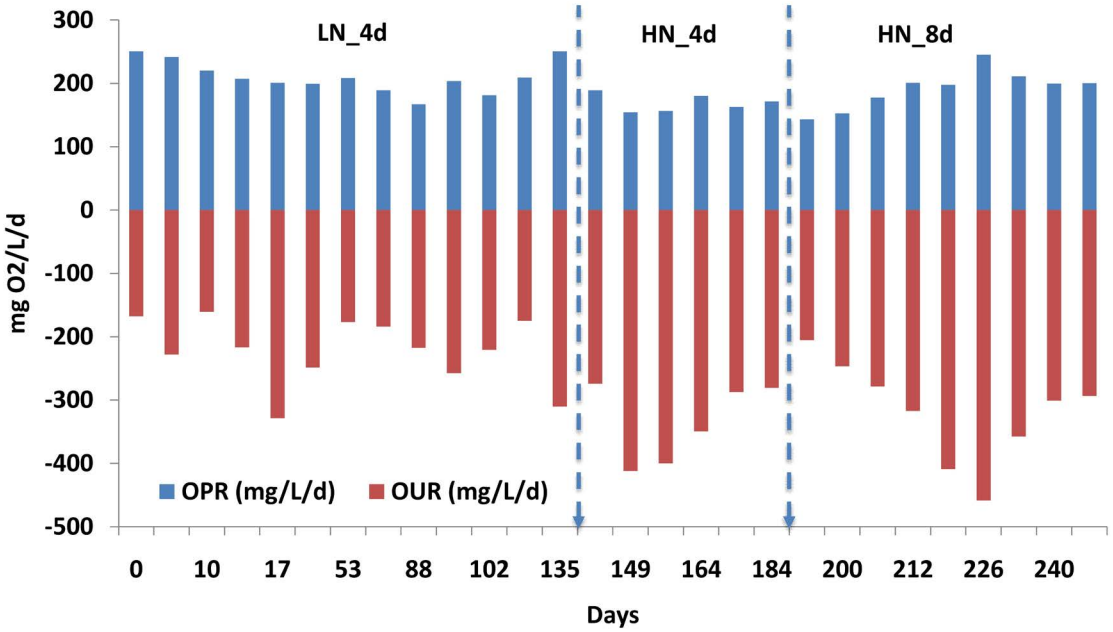

Fig. 7. Calculated OUR and OPR in HRAP reactor for different stages.

4. Conclusions and perspectives

A semi-continuous pilot HRAP system inoculated with Al-Bac biomass was operated for over 8 months to investigate the impact of high nutrient load and HRT variation on the performance of the system. Wastewater treatment efficiency, biomass productivity, and recovery were assessed. Analysis of dissolved oxygen and pH time-series by statistical decomposition was also performed to evaluate the interaction between algal and bacterial activities. The addition of high nutrient load favored bacterial growth, in particular nitrifying bacteria, but negatively impacted algal growth. High HRT and SRT resulted in a high concentration of Al-Bac biomass in the reactor. Around 99% of biomass recovery efficiency was achieved throughout the study via simple gravity settling hence reconfirming the advantage of Al-Bac biomass in enhancing settleability. High nutrient load resulted in poor TN and TP removal efficiencies and a decrease in COD removal, yet high removal of TKN and NH_4–N were obtained. HRT increase to 8 d allowed to improve COD removal: however minor impact was observed in the case of TKN, TN, and TP removals. Nitrification was identified as the main mechanism in TKN and NH_4–N removals of high nutrient load wastewater. Results from this study showed potential for a fast and simple assessment approach of algal bacterial dynamics under different influencing factors. Decomposed time-series data is promising for model validation in order to further investigate and optimize the dynamics between algae and bacteria in the HRAP system.

References

[1] W.J. Oswald, H.B. Gotaas, Photosynthesis in sewage treatment, Trans. Am. Soc. Civ. Eng., 122 (1957) 73–105.
[2] J.B.K. Park, R.J. Craggs, A.N. Shilton, Wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol., 102 (2011) 35–42.
[3] A. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, John Wiley & Sons, Oxford, UK, 2008.
[4] R. Muñoz, B. Guieysse, Algal–bacterial processes for the treatment of hazardous contaminants: a review, Water Res., 40 (2006) 2799–2815.
[5] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renewable Sustainable Energy Rev., 14 (2010) 217–232.
[6] S. Van Den Hende, H. Vervaeren, H. Saveyn, G. Maes, N. Boon, Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio, Biotechnol. Bioeng., 108 (2011) 549–558.
[7] L.A. Pham, J. Laurent, P. Bois, A. Wanko, Finding optimal algal/bacterial inoculation ratio to improve algal biomass growth with wastewater as nutrient source, Water SA, 45 (2019)624–631.
[8] Y. Su, A. Mennerich, B. Urban, Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture, Water Res., 45 (2011) 3351–3358.
[9] S. Van Den Hende, V. Beelen, G. Bore, N. Boon, H. Vervaeren, Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond, Bioresour. Technol., 159 (2014) 342–354.
[10] N. Buchanan, P. Young, N.J. Cromar, H.J. Fallowfield, Comparison of the treatment performance of a high rate algal pond and a facultative waste stabilization pond operating in rural South Australia, Water Sci. Technol., 78 (2018) 3–11.
[11] E. Posadas, A. Muñoz, M.-C. García-González, R. Muñoz, P.A. García-Encina, A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters, J. Chem. Technol. Biotechnol., 90 (2015) 1094–1101.
[12] C. Alcántara, P.A. García-Encina, R. Muñoz, Evaluation of the simultaneous biogas upgrading and treatment of centrates in a high-rate algal pond through C, N and P mass balances, Water Sci. Technol., 72 (2015) 150–157.
[13] D. García, E. Posadas, S. Blanco, G. Acién, P. García-Encina, S. Bolado, R. Muñoz, Evaluation of the dynamics of microalgae population structure and process performance during piggery

wastewater treatment in algal-bacterial photobioreactors, Bioresour. Technol., 248 (2018) 120–126.
[14] I. de Godos, S. Blanco, P.A. García-Encina, E. Becares, R. Muñoz, Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates, Bioresour. Technol., 100 (2009) 4332–4339.
[15] D.L. Sutherland, C. Howard-Williams, M.H. Turnbull, P.A. Broady, R.J. Craggs, Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol., 184 (2015) 222–229.
[16] J. González-Camejo, R. Barat, M. Pachés, M. Murgui, A. Seco, J. Ferrer, Wastewater nutrient removal in a mixed microalgae–bacteria culture: effect of light and temperature on the microalgae–bacteria competition, Environ. Technol., 39 (2018) 503–515.
[17] B. Deremince, S. Königsberger, Statistical Report of the European Biogas Association 2017, Brussels, Belgium, 2017.
[18] C. Sawatdeenarunat, D. Nguyen, K.C. Surendra, S. Shrestha, K. Rajendran, H. Oechsner, L. Xie, S.K. Khanal, Anaerobic biorefinery: current status, challenges, and opportunities, Bioresour. Technol., 215 (2016) 304–313.
[19] M. Mantovani, F. Marazzi, R. Fornaroli, M. Bellucci, E. Ficara, V. Mezzanotte, Outdoor pilot-scale raceway as a microalgae-bacteria sidestream treatment in a WWTP, Sci. Total Environ., 710 (2019) 1–12, doi: 10.1016/j.scitotenv.2019.135583.
[20] E. Ficara, A. Uslenghi, D. Basilico, V. Mezzanotte, Growth of microalgal biomass on supernatant from biosolid dewatering, Water Sci. Technol., 69 (2014) 896–902.
[21] N. Abdel-Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem, Microalgae and wastewater treatment, Saudi J. Biol. Sci., 19 (2012) 257–275.
[22] H. Hadiyanto, S. Elmore, T. Van Gerven, A. Stankiewicz, Hydrodynamic evaluations in high rate algae pond (HRAP) design, Chem. Eng. J., 217 (2013) 231–239.
[23] J.L. Mendoza, M.R. Granados, I. de Godos, F.G. Acién, E. Molina, C. Banks, S. Heaven, Fluid-dynamic characterization of real-scale raceway reactors for microalgae production, Biomass Bioenergy, 54 (2013) 267–275.
[24] P. Krebs, A.I. Stamou, J.L. García-Heras, W. Rodi, Influence of inlet and outlet configuration on the flow in secondary clarifiers, Water Sci. Technol., 34 (1996) 1–9.
[25] H. Siegrist, P. Krebs, R. Bühler, I. Purtschert, C. Rock, R. Rufer, Denitrification in secondary clarifiers, Water Sci. Technol., 31 (1995) 205–214.
[26] L.A. Pham, J. Laurent, P. Bois, A. Wanko, Impacts of operational conditions on oxygen transfer rate, mixing characteristics and residence time distribution in a pilot scale high rate algal pond, Water Sci. Technol., 78 (2018) 1782–1791.
[27] R.C. Morrow, LED lighting in horticulture, HortScience, 43 (2008) 1947–1950.
[28] R. Bouterfas, M. Belkoura, A. Dauta, The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake, Limnetica, 25 (2006) 647–656.
[29] J. Park, R. Craggs, Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition, Water Sci. Technol., 61 (2010) 633–639.
[30] ISO 15705:2002, Water Quality - Determination of the Chemical Oxygen Demand Index (ST-COD) - Small-Scale Sealed-Tube Method, 2002.
[31] ISO 5663:1984, Water Quality - Determination of Kjeldahl Nitrogen - Method after Mineralization with Selenium, 1984.
[32] AFNOR NF EN ISO 10304-1, Qualité de L'eau- Dosage des Anions Dissous par Chromatographie des Ions en Phase Liquide - Partie 1 : Dos Age du b Romure, Chlorure, Fluorure, Nitrate, Nitrite, Phosphate et Sulfate, 2009.
[33] ISO 6878:2004, Water Quality - Determination of Phosphorus - Ammonium Molybdate Spectrometric Method, 2004.
[34] NF EN 872, Qualité de L'eau - Dosage des Matières en Suspension - Méthode par Filtration sur Filtre en Fibres de Verre, 2005.
[35] AFNOR NF T 90-117, Qualité de L'eau- Dosage de la Chlorophylle a et d'un Indice Phéopigments - Méthode par Spectrométrie D'absorption Moléculaire, 1999.
[36] G. Tchobanoglous, F. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, McGraw-Hill Education, New York, USA, 2002.
[37] M.J. Crawley, The R Book, Wiley, New Jersey, USA, 2012.
[38] P. Reichert, D. Borchardt, M. Henze, W. Rauch, P. Shanahan, L. Somlyódy, P. Vanrolleghem, River water quality model no. 1 (RWQM1): II. Biochemical process equations, Water Sci. Technol., 43 (2001) 11–30.
[39] P. Young, M.J. Taylor, N. Buchanan, J. Lewis, H.J. Fallowfield, Case study on the effect continuous CO_2 enrichment, via biogas scrubbing, has on biomass production and wastewater treatment in a high rate algal pond, J. Environ. Manage., 251 (2019) 1–10, doi: 10.1016/j.jenvman.2019.109614.
[40] P. Young, M. Taylor, H.J. Fallowfield, Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment, World J. Microbiol. Biotechnol., 33 (2017) 1–13, doi: 10.1007/s11274-017-2282-x.
[41] L.T. Arashiro, I. Ferrer, D.P.L. Rousseau, S.W.H. Van Hulle, M. Garfí, The effect of primary treatment of wastewater in high rate algal pond systems: biomass and bioenergy recovery, Bioresour. Technol., 280 (2019) 27–36.
[42] J. García, B.F. Green, T. Lundquist, R. Mujeriego, M. Hernández-Mariné, W.J. Oswald, Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater, Bioresour. Technol., 97 (2006) 1709–1715.
[43] R. Craggs, D. Sutherland, H. Campbell, Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production, J. Appl. Phycol., 24 (2012) 329–337.
[44] V.V. Unnithan, A. Unc, G.B. Smith, Mini-review: a priori considerations for bacteria–algae interactions in algal biofuel systems receiving municipal wastewaters, Algal Res., 4 (2014) 35–40.
[45] G. Gutzeit, D. Lorch, A. Weber, M. Engels, U. Neis, Bioflocculant algal-bacterial biomass improves low-cost wastewater treatment., Water Sci. Technol., 52 (2005) 9–18.
[46] B. Decostere, N. Janssens, A. Alvarado, T. Maere, P. Goethals, S.W.H. Van Hulle, I. Nopens, A combined respirometer–titrimeter for the determination of microalgae kinetics: experimental data collection and modelling, Chem. Eng. J., 222 (2013) 85–93.
[47] F. García-Ochoa, E. Gómez, Mass transfer coefficient in stirred tank reactors for xanthan gum solutions, Biochem. Eng. J., 1 (1998) 1–10.
[48] J. Verbesselt, R. Hyndman, G. Newnham, D. Culvenor, Detecting trend and seasonal changes in satellite image time series, Remote Sens. Environ., 114 (2010) 106–115.
[49] Q. Béchet, A. Shilton, B. Guieysse, Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation, Biotechnol. Adv., 31 (2013) 1648–1663.
[50] A. Solimeno, J. García, Microalgae-bacteria models evolution: from microalgae steady-state to integrated microalgae-bacteria wastewater treatment models- a comparative review, Sci. Total Environ., 607–608 (2017) 1136–1150.