Enhanced nitrogen removal and energy saving in a microalgal–bacterial consortium treating real municipal wastewater

P. Foladori, S. Petrini, M. Nessenzia and G. Andreottola

ABSTRACT

The optimization of total nitrogen removal from municipal wastewater was investigated in a laboratory-scale photo-sequencing batch reactor (PSBR) operated with a mixed microalgal–bacterial consortium spontaneously acclimatized to real wastewater. No external aeration was provided in the PSBR to reduce energy consumption: oxygen was only supplied by the microalgal photosynthesis. The enhancement of total nitrogen removal was achieved through: (1) feeding of wastewater in the dark phase to provide readily biodegradable COD when oxygen was not produced, promoting denitrification; (2) intermittent use of the mixer to favor simultaneous nitrification–denitrification inside the dense flocs and to achieve 41% energy saving with respect to continuous mixing. Efficient COD removal (86 ± 2%) was observed, obtaining average effluent concentrations of 37 mg/L and 22 mg/L of total COD and soluble COD, respectively. TKN removal was 97 ± 3%, with an average effluent concentration of 0.5 ± 0.7 mg NH_4^+-N/L. Assimilation of nitrogen by heterotrophic bacteria accounted only for 20% of TKN removal, whilst the major part of TKN was nitrified. In particular, the nitrification rate was 1.9 mgN L^{-1} h^{-1} (specific rate 2.4 mgN $gTSS^{-1}$ h^{-1}), measured with dissolved oxygen near zero, when the oxygen demand was higher than the oxygen produced by photosynthesis. Total nitrogen of 6.3 ± 4.4 mgN/L was measured in the effluent after PSBR optimization.

Key words | microalgae, microalgal–bacterial consortia, nitrification–denitrification, nitrogen removal, photobioreactor, real wastewater

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INTRODUCTION

The treatment of real wastewater with microalgal–bacterial consortia has gained increasing attention in the last few years because the synergistic effects of microalgae and heterotrophic bacteria can be exploited for carbon and nitrogen removal through nitrification–denitrification, ensuring a significant energy saving (Su *et al.* 2012; Zhang *et al.* 2012; He *et al.* 2013; Abinandan & Shanthakumar 2015; Wang *et al.* 2016b; Arcila & Buitrón 2017; Quijano *et al.* 2017).

However, when ammonium removal occurs with nitrifiers in activated sludge, a huge amount of electrical energy is required to supply air in aerobic bioreactors. This energy consumption, represents up to half of the energy expenditure of a wastewater treatment plant (WWTP) based on activated sludge. In particular, considering the annual energy consumption by the air supply in aerated tanks in the range of 4.7–28 kWh PE^{-1} y^{-1} (Foladori *et al.* 2015), a medium-size WWTP (20,000 population equivalent, PE) may consume 94–560 MWh/y, which corresponds to an annual cost of 11,000–64,000 €/y (average EU electricity price for industrial consumers of 0.114 €/kWh; Eurostat 2017).

These figures emphasize the need to explore new alternatives aimed at reducing energy consumption and associated costs of wastewater treatment in WWTPs. In this context, the development of heterogeneous

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microalgal–bacterial consortia offers the possibility of moving towards quasi-zero-energy consumption; in particular, the oxygen produced by photosynthetic microorganisms may support the oxidation of organic matter and ammonium by heterotrophic and nitrifying bacteria (Van den Hende *et al.* 2014). Suspended-biomass reactors operating as photo-sequencing batch reactors (PSBR) represent a common configuration for the laboratory-scale implementation of microalgal–bacteria consortia (Arcila & Buitrón 2016; Wang *et al.* 2016b) because of the easy setup with small pumps and timers.

With regard to nitrogen removal in microalga–bacteria consortia, the main mechanisms are biomass assimilation, nitrification–denitrification and, when high pH values are reached, ammonia volatilization (Delgadillo-Mirquez *et al.* 2016; Wang *et al.* 2016b). González-Fernández *et al.* (2011) observed that microalgal assimilation of nitrogen is frequently overestimated. Su *et al.* (2011), treating municipal wastewater with a mixed population of microalgae and bacteria, observed that denitrification was considered to be a possible removal mechanism, while ammonia volatilization was excluded due to the pH being lower than 8.5.

In spite of the advantage of oxygen production by photosynthetic microorganisms, dissolved oxygen (DO) released in the bulk liquid may become excessive and may inhibit the transformation of nitrates into nitrogen gas through heterotrophic denitrification, which requires strictly anoxic conditions. Thus, it may be difficult to meet the legal requirements for total nitrogen in the effluent if the operational strategies in the microalgal system are not optimized. Wang *et al.* (2015) achieved 90% of nitrogen removal by alternating light and dark periods and without artificial aeration, but to promote denitrification during the dark period, an organic carbon source was added.

The present experimental study focuses on the optimization of total nitrogen removal in a laboratory-scale PSBR fed with real wastewater and operated with a mixed consortium of microalgae, cyanobacteria and bacteria. The phases in the PSBR were feeding + react + settling + draw, but no idle phase was included in the cycles. The length of the phases and the photoperiod (light/dark) were combined so as to affect the duration of the aerobic and anoxic periods and promote nitrification and denitrification, without the need for an additional external carbon source. This paper contributes to the optimization of a PSBR, in terms of enhancement of nitrogen removal, reduction of the time of treatment, and energy saving where possible, thus improving the global sustainability of the system.

MATERIALS AND METHODS

Influent wastewater

Influent municipal pre-settled wastewater was collected after the primary settler of the Trento Nord WWTP (100,000 PE) and fed in to a laboratory-scale photobioreactor. No filtration of the influent wastewater was performed before feeding in to the reactor. This was to allow the microorganisms naturally present in the influent wastewater to enter the reactor, thus significantly affecting the composition of the microalgal–bacterial consortium developed in the system. Table 1 shows the concentrations of the main parameters in the influent pre-settled wastewater: chemical oxygen demand (COD), soluble COD (sCOD), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), TN, total phosphorus (TP), orthophosphate phosphorus (PO_4^{3-}-P) and total suspended solids (TSS). The average concentrations are in agreement with typical values expected in pre-settled wastewater (Tchobanoglous *et al.* 2003).

Photo-sequencing batch reactor

The PSBR setup consisted of a cylindrical bench-scale reactor made of Pyrex glass with diameter of 0.13 m, height of 0.29 m and a working volume of 2 L (Colaver, Italy) not sealed off from atmosphere (Figure 1(a)). The system was equipped with:

- peristaltic pumps (Kronos Seko, Italy) for pumping the influent and discharging the effluent; 0.7 L of influent wastewater was fed per cycle;
- cool-white lamp, 8 LEDs × 0.5 W, 0.18 m high and 0.065 m wide (Orion, Italy), arranged on one side of the PSBR;

Table 1 | Characterization of influent pre-settled wastewater (average ± standard deviation; number of samples = 30)

Parameter	Concentration (mg/L)
COD	257 ± 91
sCOD	120 ± 42
TKN	55 ± 20
NH_4^+-N	50 ± 14
NO_2^--N	0.1 ± 0.1
NO_3^--N	1.2 ± 0.9
TN	54 ± 22
TP	4.8 ± 1.5
PO_4^{3-}-P	2.8 ± 0.9
TSS	184 ± 122

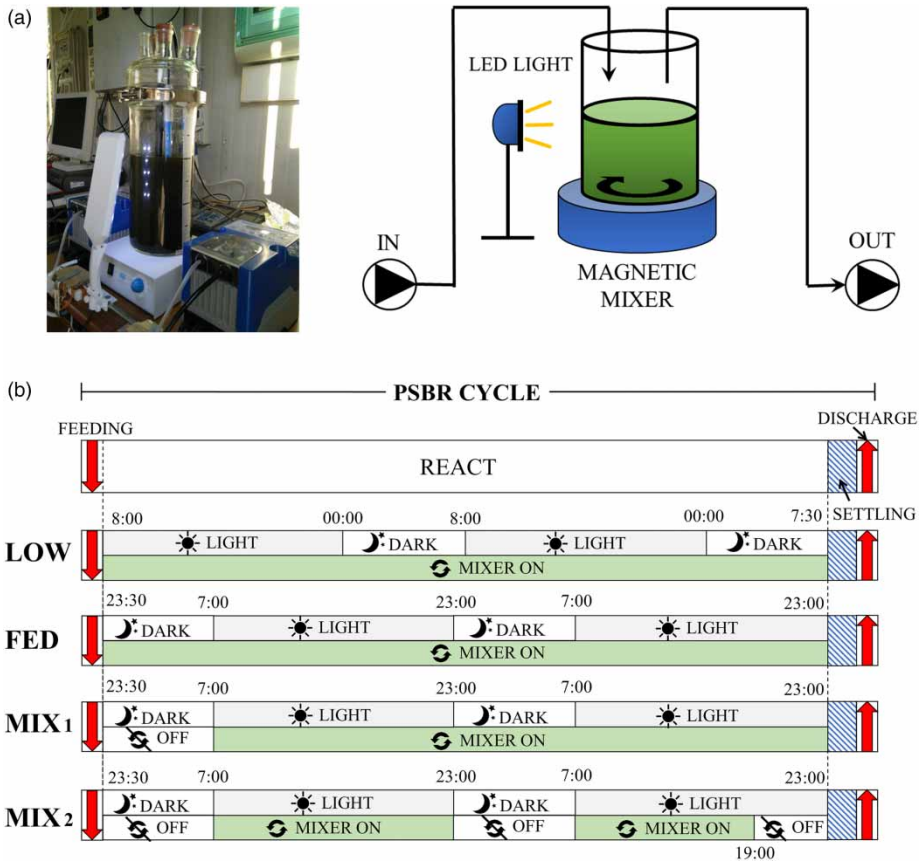

Figure 1 | (a) Laboratory-scale PSBR; (b) operating cycles during the four regimes (LOW, FED, MIX_1, MIX_2).

- magnetic mixer set at 200 rpm (AGE, Velp, Italy) used to maintain the biomass in suspension during the react phase, but avoiding excessive turbulence and reoxygenation from the air;
- timers (TR 612 top2, Theben, Germany) to control on/off of pumps, lamp and mixer.

A light intensity of 30 μmol m^{-2} s^{-1} (expressed as photosynthetically active radiation, PAR) was measured inside the reactor near the top of the liquid surface, at the most exposed point. Light originated from solar light penetration in to the laboratory and the lamp, but the reactor was never exposed to direct sunlight. This light intensity is in the same order of magnitude as other studies in the literature (for example 63 μmol m^{-2} s^{-1} in Karya *et al.* 2013; 90 μmol m^{-2} s^{-1}, in Mennaa *et al.* 2015) but lower than the PAR in outdoor plants which surpasses 200 μmol m^{-2} s^{-1} (light irradiances that saturate photosynthesis). It is worth noting that no external aeration was provided in the PSBR (in order to reduce energy consumption and move towards a zero-energy system); the oxygen available in the reactor was supplied only by microalgal photosynthesis.

The temperature of the mixed liquor during the experiment was 22.8 °C on average.

An acclimatized microalgal–bacterial consortium that had spontaneously developed over more than a year in the PSBR was employed in this study, which was carried out from January to July 2017. Microscopic observations of the biomass showed the presence of *Chlorella*, diatoms, many filamentous cyanobacteria and heterotrophic bacteria embedded in flocs.

Operational strategy in the PSBR

The typical PSBR cycle was based on the following phases: (1) feeding, with duration of 5 min; (2) react, 47.5 hours; (3) settling, 30 min; (4) draw, 5 min. The photoperiod was 16 hours of light + 8 hours of dark and the sequence of the light and dark periods is summarized in Figure 1(b). A constant photoperiod using artificial light allowed us to control the photoperiod and understand the process clearly. The sequence of light and dark causes the alternation of high and low oxygen in the bulk liquid and thus the alternation of aerobic and anoxic conditions, which can be

advantageously exploited for optimizing nitrogen removal from wastewater.

In order to enhance total nitrogen removal and save energy in the PSBR, four different operational regimes were applied during the experiment (Figure 1(b)):

- LOW: feeding at the beginning of the light phase (at 8:00) and continuous mixing during the react phase. This basic operational regime was used as the control/starting point, but it did not meet the EU discharge limits in sensitive areas for total nitrogen (15 mg N/L for 10,000 < PE < 100,000; 10 mg N/L for PE > 100,000), requiring progressive optimizations.
- FED: feeding at the beginning of the dark phase (at 23:30) and continuous mixing in the react phase; the aim was to create an anoxic phase at the beginning of the cycle (to exploit readily biodegradable COD, RBCOD, and the anoxic conditions of the influent wastewater) in order to promote denitrification and thus to enhance total nitrogen removal.
- MIX_1: feeding at the beginning of the dark phase (at 23:30) with the mixer off until the beginning of the light phase (at 07:00); the aim was to create an anoxic phase at the beginning of the cycle and to reduce the energy consumption by stopping the mixing for 7.5 hours in the cycle (16% energy saving).
- MIX_2: feeding at the beginning of the dark phase (at 23:30) with the mixer off during all the dark phases and before the end of the react phase (at 19:00); the aim was to create longer anoxic conditions to enhance denitrification and to reduce the energy consumption by stopping the mixing for 19.5 hours in the cycle (41% energy saving).

Due to the good settleability of the biomass in all the operational regimes, the duration of the settling phase (30 min) was appropriate and remained unchanged.

Analytical methods

The chemical parameters COD, sCOD, TKN, NH_4^+-N, NO_2^--N, NO_3^--N, TP, PO_4^{3-}-P and TSS were analyzed in the influent and effluent wastewater according to *Standard Methods* (APHA 2012). Influent TN was estimated as the sum of TKN and NO_x-N. The parameter sCOD was measured after filtration of the sample through a 0.45 µm membrane. Fractionation of COD in the influent wastewater aimed at distinguishing soluble and particulate biodegradable COD was performed by respirometry (Vanrolleghem *et al.* 1999; Ziglio *et al.* 2001). TSS in the mixed liquor (APHA 2012) was measured to determine the concentration of the microalgal–bacterial consortium in the PSBR. DO and temperature were measured with a Multi3410 meter and an FDO®925 sensor (WTW, Germany). Oxidation–reduction potential (ORP) and pH were measured continuously (every 10 min) with a pH3310 meter coupled with the electrodes Sentix®ORP and Sentix®41, respectively (all from WTW, Germany). PAR was measured with a quantum sensor SQ-520 (Apogee Instruments, USA). Microscopic observations were performed using a Nikon Optiphot EFD-3 Microscope (Nikon, Japan) for the morphological characterization of the microalgal–bacterial consortium.

Track studies

Track studies were performed in the PSBR to measure the dynamics of the N-forms during a typical cycle. Samples were collected every hour, then filtered and finally analyzed for NH_4^+-N, NO_2^--N and NO_3^--N. The volumetric removal of NH_4^+-N (or the production of NO_3^--N) expressed as mg N L^{-1} h^{-1} was estimated using the slope of the straight line that interpolates the experimental concentrations over time during the track study. The corresponding specific rate, expressed as mg N g TSS^{-1} h^{-1}, was obtained by dividing the volumetric rate by the TSS concentration in the mixed liquor. The removal rate was also expressed per unit of surface, which was useful for appreciating the footprint of the system, taking into account the height of mixed liquor in the reactor.

Statistical analysis

A Student's t test, producing a p value, was used to evaluate the significant difference in results. Significance was assumed at $p < 0.05$.

RESULTS AND DISCUSSION

PSBR ensures robust and efficient COD removal (>85%) in all the operational regimes

The profiles of COD and sCOD concentrations in the influent and effluent wastewater during the 6-month experiment are shown in Figure 2. Although large variations of COD concentration in real wastewater were common (257 ± 91 mg/L), the average concentrations in the effluent were 37 ± 7 mg COD/L (min–max range equal to 21–54 mg COD/L), without statistically significant variations among the operational regimes. These results largely met

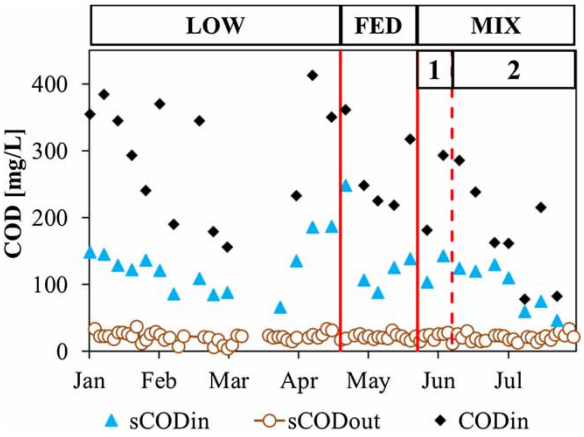

Figure 2 | Profiles of COD and sCOD in the influent and effluent wastewater.

the EU limit for discharge (125 mg COD/L, according to EU Directive 91/271/EEC). The effluent total COD was measured analytically or calculated by summing the soluble COD (22 mg sCOD/L on average) and the particulate COD estimated from effluent TSS concentration, VSS/TSS ratio of mixed liquor (that was 0.80 on average) and the conversion factor 1.42 g COD/gVSS (volatile suspended solids).

High removal efficiency of COD was achieved in all the PSBR operational regimes (86 ± 2% on average), indicating that the variations of influent load and stopping the mixing did not significantly affect organic matter removal. The PSBR appeared to be a very robust process to treat real municipal wastewater. For comparison, COD removal >70% was found by Arango *et al.* (2016) in the treatment of municipal wastewater with an average influent sCOD of 410 mg/L in a PSBR inoculated with microalgae and activated sludge. COD removal efficiency >80% was found in a PSBR for the treatment of synthetic wastewater with 420 mg COD/L (Gutzeit *et al.* 2005). The removal of COD from municipal wastewater by microalgal–bacterial consortia has been reported to be a very effective process (Gutzeit *et al.* 2005; Su *et al.* 2012; Wang *et al.* 2016a).

The fractionation of influent COD permits an understanding of the composition of the effluent COD (Henze *et al.* 2008):

- particulate inert COD ($X_I = 46 \pm 16$ mg/L; 18% of COD) and particulate biodegradable COD ($X_S = 100 \pm 36$ mg/L; 39%) can be flocculated on suspended solids;
- soluble biodegradable COD ($S_S = 95 \pm 34$ mg/L; 37%) is rapidly biodegraded;
- soluble inert COD ($S_I = 16 \pm 5$ mg/L; 6% of COD) cannot be removed and thus it leaves the system in the effluent.

This COD fractionation suggests that the effluent soluble COD (22 ± 6 mg/L) was mainly composed of the soluble inert fraction of the influent wastewater (S_I) which passed unchanged through the PSBR, while the rest may have been soluble microbial products (SMPs) originating from substrate metabolism and biomass decay (Azami *et al.* 2012).

The particulate COD in the effluent was associated with some suspended solids floating and leaving the system during discharge. The effluent TSS concentration was around 13 mg TSS/L during the whole experiment, indicating very good sedimentation of the biological flocs developed in the PSBR.

With regard to the removal of nutrients in the whole experiment, average P concentration decreased from 4.8 ± 1.5 mg/L in the influent to 2.5 ± 0.8 mg/L in the effluent (average removal efficiency of 47 ± 19%), while N removal is discussed in depth in the following sections.

TKN removal >95% occurs in the PSBR at near-zero DO

The influent TKN (55 ± 20 mg TKN/L) was almost entirely removed in the PSBR, leading to effluent concentrations of 1.5 ± 2.1 mg TKN/L, 0.5 ± 0.7 mgNH_4^+-N/L and 0.1 ± 0.4 mg NO_2^--N/L.

There are three ways in which TKN and ammonium can be transformed by the microalgal–bacterial consortium: ammonia volatilization, assimilation by biomass and nitrification. In particular, volatilization was negligible in this PSBR because the pH in the reactor was 7.6 ± 0.3 on average during the whole experiment, and ammonia volatilization requires relatively high pH values. Nitrogen is also assimilated and incorporated into biomass through cell synthesis during the growth of heterotrophs and, to a lesser extent, autotrophs (Tchobanoglous *et al.* 2003). The real amount of N removed by net microbial biomass growth was estimated taking into account sludge production, which was 0.03 g TSS/d. Considering the specific content of N in biomass (0.10 g N/g VSS), an amount of 2.4 mg N/L was used daily for the net growth. It accounts for about 4% of the TKN transformed in the system. This highlights that sludge production and nutrient uptake in PSBRs applied to the highly efficient treatment of real municipal wastewater may be significantly lower than that expected in PSBRs specifically designed to maximize algal growth.

The N mass balance reveals that the major role in TKN removal in the PSBR was played by autotrophic nitrification. Karya *et al.* (2013) also observed that nitrification accounted for more than 80% of ammonium removal during the

treatment of synthetic artificial wastewater in an open photobioreactor inoculated with nitrifying activated sludge and a pure culture of *Scenedesmus* sp.

The very low concentration of NH_4^+-N in the effluent wastewater (<1.0 mg/L in 89 samples, with the exception of only three cases) demonstrates that nitrification was complete during the 6-month experiment and not negatively affected by the different operational regimes implemented in the PSBR. As shown in Figure 3, the average TKN removal efficiency was 97 ± 3% and very stable during the whole period, even when the influent TKN concentration reached very high values of around 100 mg TKN/L (Figure 3).

On the basis of these considerations, the nitrification kinetic in the PSBR reactor was investigated in depth. Track studies were carried out in the reactor after the addition of influent wastewater with a known amount of ammonium and the time profiles of NH_4^+-N, NO_2^--N, NO_3^--N were measured. The results obtained in a typical track study carried out during the light phase of the LOW operational regime (used as control) are shown in Figure 4. The decrease of ammonium occurred rapidly and the initial concentration of 14 mg NH_4^+-N/L was completely removed in less than 7 hours, corresponding to a nitrification rate of 1.9 mg N L^{-1} h^{-1}. Considering that the biomass in the PSBR was 0.8 g TSS/L, a specific nitrification rate of 2.4 mg NH_4^+-N g TSS^{-1} h^{-1} was calculated. Similar results for nitrification kinetics were found during the other operational regimes (FED, MIX_1, MIX_2), which did not significantly affect ammonium removal.

Considering the hourly nitrification rate, a daily rate of 30 mg NH_4^+-N L^{-1} d^{-1} can be calculated for a daily photoperiod of 16/24 hours, which corresponds to a surface removal rate of 5.4 g NH_4^+-N m^{-2} d^{-1} (considering the reactor's diameter of 0.13 m, top surface area of 0.0133 m^2 and volume of 0.0024 m^3). This specific value gives an approximate estimate of the footprint. For a comparison, 10.2 g NH_4^+-N m^{-2} d^{-1} was found in a flat PSBR with irradiance an order of magnitude higher than in this experiment (Rada-Ariza *et al.* 2017).

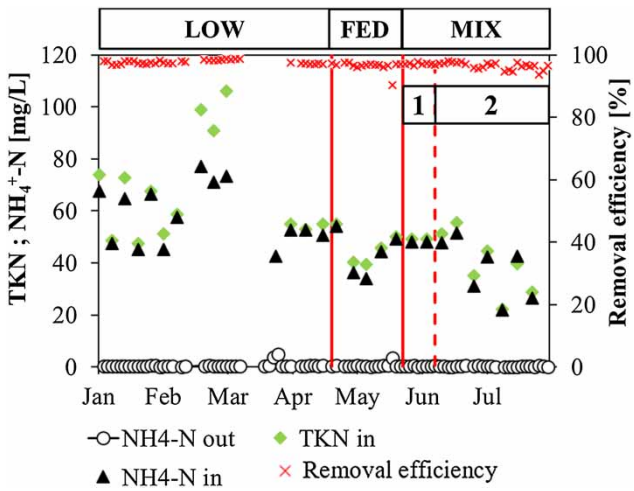

Figure 3 | Profiles of TKN and NH_4^+-N in the influent and effluent wastewater, and removal efficiency of TKN.

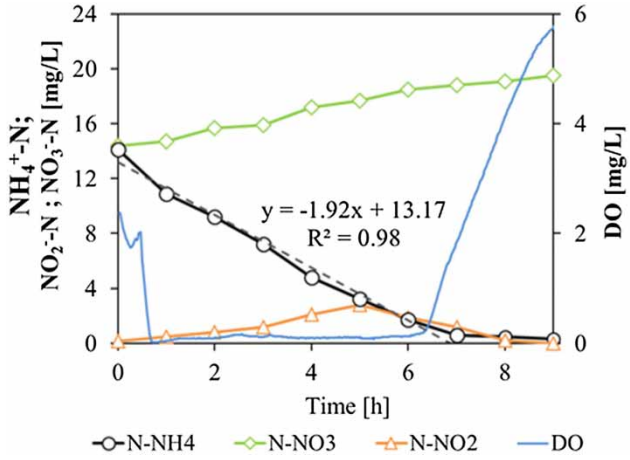

Figure 4 | Track study of NH_4^+-N, NO_3-N and NO_2-N in the PSBR to evaluate nitrification kinetics during the light phase.

The specific value of 2.4 mg N $gTSS^{-1}$ h^{-1} found for the microalgal–bacterial consortium is similar to the specific nitrification rate reported in the literature for activated sludge systems (Tchobanoglous *et al.* 2003). However, the important difference is that the microalgal–bacterial consortium works with a limited TSS concentration (around 1 g TSS/L) to allow light to penetrate in the biomass, while activated sludge works at 4 g TSS/L. This results in a significant difference in the volumetric nitrification rate of microalgal consortia and activated sludge. However, a further investigation is needed at a real scale to evaluate the volumetric nitrification rate of microalgal–bacteria consortia under more realistic conditions.

From Figure 4 it can be observed that the decrease of ammonium occurred steadily over a long period (>5 hours) with DO concentrations near zero. Since no external aeration was provided to the PSBR, only photosynthetic activity was responsible for oxygen production during the light phase. In the presence of NH_4^+, the oxygen demand required for nitrification surpassed the amount produced by photosynthesis, resulting in a near-zero DO concentration. Then, when ammonium was completely depleted, the DO concentration increased gradually, reaching values above 5 mg O_2/L.

Concerning the profile of NO_3^--N, the initial concentration of 14 mg N/L increased to 19.5 mg N/L at the end of the track study, indicating that only 40% of the ammonium removed was found as nitrates. The explanation of this low increase in nitrates is that simultaneous nitrification–denitrification occurred in this PSBR. The availability of soluble COD, which decreased from 121 mg/L in the influent wastewater to 17 mg/L at the end of the track study, may support denitrification. Analogous observations were found by Tiron *et al.* (2015) in activated algae granules treating low-strength wastewater in a PSBR. In the present reactor, dense clusters of microalgae, bacteria and abiotic solids were also observed, and this may contribute to initiating conditions for partial denitrification.

Although the near-zero DO phase may suggest the absence of oxygen, it must not be confused with a completely anoxic phase, because photosynthesis occurred and oxygen was continuously produced in the system, but there was not enough oxygen to satisfy the requirements of the biomass. The advantage of nitrification in municipal wastewater by exploiting the oxygen produced in a microalgal–bacterial consortium, without the need of artificial aeration, may offer very powerful opportunities in the evolution of WWTPs. However, further research is needed to prove the feasibility of the process under real conditions in order to evaluate the influence of the main parameters' variations (e.g. light supply, mass transfer and temperature).

Total nitrogen <10 mgN/L in the effluent through the enhancement of denitrification in the PSBR

The LOW operational regime, used as the control, produced a concentration of TN in the effluent wastewater in the range 6.8–40.0 mg N/L, which was over the limits of 10–15 mg N/L (as an annual average) for discharge in sensitive areas imposed by EU Directive 91/271/EEC (Figure 5). The high TN concentration in the effluent, largely composed by nitrates, was the result of: (1) slight assimilation of N by biomass and (2) only partial denitrification.

To enhance TN removal, efforts aimed at increasing the assimilation of N by pure microalgae do not seem to be so effective, because assimilation permits moderate N removal rates, often requires a longer reaction time and thus larger volumes for the treatment of wastewater.

The great advantage of using microalgal–bacterial consortia instead of pure microalgal strains is that bacterial nitrification can be exploited to speed up ammonium removal. Then a higher TN removal can be achieved by ensuring a denitrification step (Rada-Ariza *et al.* 2017).

In our case, the microalgal–bacterial consortium removed 30 mg N L^{-1} d^{-1} for a daily photoperiod of 16/24 hours, which was noticeably higher than those found by Álvarez-Díaz *et al.* (2017) (N removal of 6.6 mg N L^{-1} d^{-1}) treating secondary effluents under 14/24 hours of light with *Chlorella sorokiniana*. A similar N removal (6.63 mg N L^{-1} d^{-1}) was reported by Cabanelas *et al.* (2013) in the case of *Chlorella vulgaris* treating urban wastewater under 14/24 hours of light.

Concerning denitrification, there could still be room for improvement, when suitable conditions were applied in the PSBR. In the LOW operational regime the ORP profile (data not shown) assumed negative values only for 2–4 hours per cycle after the feeding phase, not long enough to create the conditions for full denitrification in the system. Therefore, the enhancement of TN removal was investigated through the optimization of the denitrification process, but without worsening the effluent quality in terms of COD and NH_4^+-N. This was achieved with the following modifications:

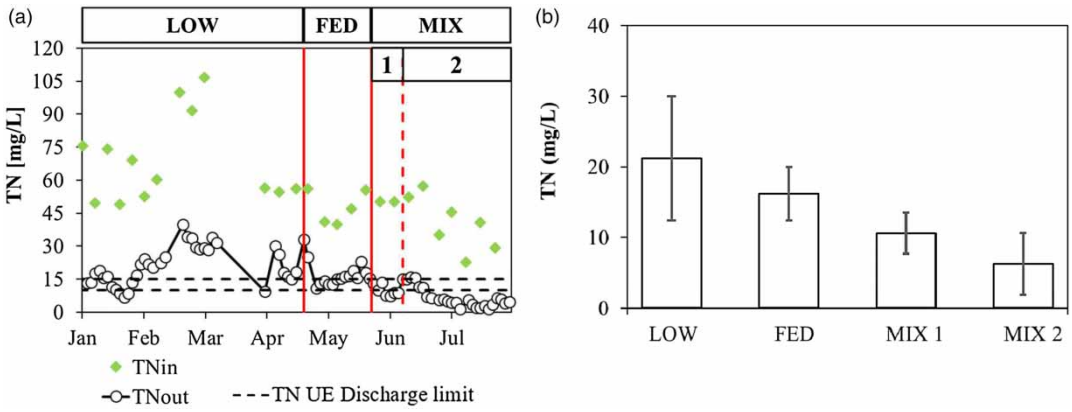

Figure 5 | (a) Profiles of total nitrogen in the influent and effluent wastewater compared with the discharge limit (10–15 mgN/L; EU Directive 91/271/EEC); (b) average effluent concentration and standard deviation for each operational regime.

1. The time of feeding (FED operational regime): wastewater was fed during the dark period instead of the light period, in order to provide readily biodegradable COD, required for denitrification, during the dark period when oxygen is consumed but not produced.
2. The intermittent use of the mixer (MIX operational regime): switched on during the light phases, when the biomass requires complete exposure to the light for photosynthesis, and switched off during the night, to avoid turbulence, favor denitrification inside flocs and energy saving; in this phase the biomass settled but the process was not negatively affected.

As shown in Figure 5, the application of the FED and MIX operational regimes resulted in a gradually decreasing profile of TN. The average TN concentration in the effluent was 21 ± 8.8 mg N/L in the LOW operational regime (not optimized), 16.2 ± 3.8 mg N/L in the FED regime, and 10.6 ± 2.9 mg N/L in the MIX_1 regime, reaching the lowest average value of 6.3 ± 4.4 mg N/L in the MIX_2 regime, which met the EU regulation limits. The enhancement of total N removal due to the progressive optimization of the PSBR was evident only when the MIX regimes were applied. In fact, no statistically significant difference was observed between the LOW and FED regimes, while effluent TN decreased significantly in the MIX_1 and MIX_2 regimes ($p < 0.05$).

The decrease of TN occurred along with the extension of the period with negative ORP in the cycle. ORP values in the range −50 to − 200 mV, which are favorable for denitrification, were measured for periods of 7–8 hours during the night in the MIX_2 regime. In this case, the mixer was switched off in the light phase (at 19:00, for 4 hours before settling and discharge), in order to move toward anoxic conditions before feeding, thus favoring denitrification at the beginning of the subsequent cycle.

CONCLUSIONS

The total nitrogen removal in a PSBR fed with real wastewater and operated with a mixed microalgal–bacterial consortium was investigated and optimized to achieve effluent TN concentrations in accordance with EU requirements for discharge in sensitive areas.

COD and TKN removal in the whole experiment were $86 \pm 2\%$ and $97 \pm 3\%$, producing an average effluent concentration of 37 mg COD/L and 0.5 ± 0.7 mg NH_4^+-N/L. In spite of long periods with DO near zero during the PSBR cycle, due to an oxygen demand higher than photosynthesis production, the nitrification rate was 1.9 mg N L^{-1} h^{-1}, corresponding to a specific rate of 2.4 mg N g TSS^{-1} h^{-1}. PSBR optimization was aimed at enhancing denitrification through the feeding during the dark and the intermittent use of the mixer; in this way, the total nitrogen in the effluent during the MIX_2 regime reached 6.3 ± 4.4 mg N/L.

In conclusion, the PSBR allowed the nitrification of municipal wastewater under laboratory conditions without the need for artificial aeration and with an important energy saving, while simultaneous denitrification was exploited to enhance total nitrogen removal. However, considering the ability of microalgae or associated bacteria to synthesize N_2O, further research is needed to couple the nitrogen balance with a greenhouse gas mass balance.

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REFERENCES

Abinandan, S. & Shanthakumar, S. 2015 Challenges and opportunities in application of microalgae (*Chlorophyta*) for wastewater treatment: a review. *Renewable and Sustainable Energy Reviews* **52**, 123–132. http://dx.doi.org/10.1016/j.rser.2015.07.086.

Álvarez-Díaz, P. D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez, M. C. & Perales, J. A. 2017 Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production. *Algal Research* **24**, 477–485. http://dx.doi.org/10.1016/j.algal.2017.02.006.

APHA 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Arango, L., Cuervo, F. M., González-Sánchez, A. & Buitrón, G. 2016 Effect of microalgae inoculation on the start-up of microalgae–bacteria systems treating municipal, piggery and digestate wastewaters. *Water Science & Technology* **73** (3), 687–696. doi:10.2166/wst.2015.544.

Arcila, J. S. & Buitrón, G. 2016 Microalgae–bacteria aggregates: effect of the hydraulic retention time on the municipal wastewater treatment, biomass settleability and methane potential. *Journal of Chemical Technology and Biotechnology* **91**, 2862–2870. doi 10.1002/jctb.4901.

Arcila, J. S. & Buitrón, G. 2017 Influence of solar irradiance levels on the formation of microalgae-bacteria aggregates for

municipal wastewater treatment. *Algal Research* **27**, 190–197. https://doi.org/10.1016/j.algal.2017.09.011.

Azami, H., Sarrafzadeh, M. H. & Mehrnia, M. R. 2012 Soluble microbial products (SMPs) release in activated sludge systems: a review. *Iranian Journal of Environmental Health Science and Engineering* **9** (1), 30. doi:10.1186/1735-2746-9-30.

Cabanelas, I. T. D., Ruiz, J., Arbib, Z., Chinalia, F. A., Garrido-Pérez, C., Rogalla, F., Nascimento, I. A. & Perales, J. A. 2013 Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. *Bioresource Technology* **131**, 429–436. http://dx.doi.org/10.1016/j.biortech.2012.12.152.

Delgadillo-Mirquez, L., Lopes, F., Taidi, B. & Pareau, D. 2016 Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. *Biotechnology Reports* **11**, 18–26. http://dx.doi.org/10.1016/j.btre.2016.04.003.

EU Directive 91/271/EEC 1991 Council Directive of 21 May 1991 concerning urban waste water treatment.

Eurostat, Statistics Explained: Electricity price statistics. http://ec.europa.eu/eurostat/statistics-explained/index.php/Electricity_price_statistics (accessed 26 September 2017).

Foladori, P., Vaccari, M. & Vitali, F. 2015 Energy audit in small wastewater treatment plants: methodology, energy consumption indicators, and lessons learned. *Water Science & Technology* **72** (6), 1007–1015. doi:10.2166/wst.2015.306.

González-Fernández, C., Molinuevo-Salces, B. & Cruz García-González, M. 2011 Nitrogen transformations under different conditions in open ponds by means of microalgae–bacteria consortium treating pig slurry. *Bioresource Technology* **102**, 960–966. doi:10.1016/j.biortech.2010.09.052.

Gutzeit, G., Lorch, D., Weber, A., Engels, M. & Neis, U. 2005 Bioflocculent algal-bacterial biomass improves low-cost wastewater treatment. *Water Science & Technology* **52** (12), 9–18.

He, P. J., Mao, B., Lü, F., Shao, L. M., Lee, D. J. & Chang, J. S. 2013 The combined effect of bacteria and *Chlorella vulgaris* on the treatment of municipal wastewaters. *Bioresource Technology* **146**, 562–568. http://dx.doi.org/10.1016/j.biortech.2013.07.111.

Henze, M., van Loosdrecht, M. C. M., Ekama, G. A. & Brdjanovic, D. 2008 *Biological Wastewater Treatment: Principles, Design and Modelling*. IWA Publishing, London, UK, pp. 526.

Karya, N. G. A. I., van der Steen, N. P. & Lens, P. N. L. 2013 Photo-oxygenation to support nitrification in an algal–bacterial consortium treating artificial wastewater. *Bioresource Technology* **134**, 244–250. http://dx.doi.org/10.1016/j.biortech.2013.02.005.

Mennaa, F. Z., Arbib, Z. & Perales, J. A. 2015 Urban wastewater treatment by seven species of microalgae and an algal bloom: biomass production, N and P removal kinetics and harvestability. *Water Research* **83**, 42–51. http://dx.doi.org/10.1016/j.watres.2015.06.007.

Quijano, G., Arcila, J. S. & Buitrón, G. 2017 Microalgal-bacterial aggregates: applications and perspectives for wastewater treatment. *Biotechnology Advances* **35**, 772–781. http://dx.doi.org/10.1016/j.biotechadv.2017.07.003.

Rada-Ariza, A. M., Lopez-Vazquez, C. M., van der Steen, N. P. & Lens, P. N. L. 2017 Nitrification by microalgal-bacterial consortia for ammonium removal in flat panel sequencing batch photo-bioreactors. *Bioresource Technology* **245**, 81–89.

Su, Y., Mennerich, A. & Urban, B. 2011 Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research* **45**, 3351–3358. doi:10.1016/j.watres.2011.03.046.

Su, Y., Mennerich, A. & Urban, B. 2012 Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. *Bioresource Technology* **150**, 67–73. doi:10.1016/j.biortech.2011.11.113.

Tchobanoglous, G., Burton, F. L. & Stensel, H. D. 2003 *Wastewater Engineering: Treatment and Reuse*, 4th edn. McGraw-Hill Metcalf & Eddy Inc., Boston.

Tiron, O., Bumbac, C., Patroescu, I. V., Badescu, V. R. & Postolache, C. 2015 Granular activated algae for wastewater treatment. *Water Science & Technology* **71** (6), 832–839. doi:10.2166/wst.2015.010.

Van Den Hende, S., Carré, E., Cocaud, E., Beelen, V., Boon, N. & Vervaeren, H. 2014 Treatment of industrial wastewaters by microalgal bacterial flocs in sequencing batch reactors. *Bioresource Technology* **161**, 245–254. http://dx.doi.org/10.1016/j.biortech.2014.03.057.

Vanrolleghem, P. A., Spanjers, H., Petersen, B., Ginestet, P. & Takacs, I. 1999 Estimating (combinations of) activated sludge Model No. 1 parameters and components by respirometry. *Water Science & Technology* **39** (1), 195–214.

Wang, M., Yang, H., Ergas, S. J. & van der Steen, P. 2015 A novel shortcut nitrogen removal process using an algal–bacterial consortium in a photo-sequencing batch reactor (PSBR). *Water Research* **87**, 38–48.

Wang, L., Liu, J., Zhao, Q., Wei, W. & Sun, Y. 2016a Comparative study of wastewater treatment and nutrient recycle via activated sludge, microalgae and combination systems. *Bioresource Technology* **211**, 1–5. http://dx.doi.org/10.1016/j.biortech.2016.03.048.

Wang, Y., Ho, S., Cheng, C., Guo, W., Nagarajan, D., Ren, N., Lee, D. & Chang, J. 2016b Perspectives on the feasibility of using microalgae for industrial wastewater treatment. *Bioresource Technology* **222**, 485–497. http://dx.doi.org/10.1016/j.biortech.2016.09.106.

Zhang, Y., Su, H., Zhong, Y., Zhang, C., Shen, Z., Sang, W., Yan, G. & Zhou, X. 2012 The effect of bacterial contamination on the heterotrophic cultivation of *Chlorella pyrenoidosa* in wastewater from the production of soybean products. *Water Research* **46**, 5509–5516. http://dx.doi.org/10.1016/j.watres.2012.07.025.

Ziglio, G., Andreottola, G., Foladori, P. & Ragazzi, M. 2001 Experimental validation of a single-OUR method for wastewater RBCOD characterisation. *Water Science & Technology* **43** (11), 119–126.

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