Modelling shortcut nitrogen removal from wastewater using an algal–bacterial consortium

Larissa T. Arashiro, Angelica M. Rada-Ariza, Meng Wang, Peter van der Steen and Sarina J. Ergas

ABSTRACT

A shortcut nitrogen removal process was investigated for treatment of high ammonium strength wastewater using an algal–bacterial consortium in photo-sequencing batch reactors (PSBRs). In this process, algae provide oxygen for nitritation during the light period, while denitritation takes place during the dark (anoxic) period, reducing overall energy and chemical requirements. Two PSBRs were operated at different solids retention times (SRTs) and fed with a high ammonium concentration wastewater (264 mg NH_4^+-N L^{-1}), with a '12 hour on, 12 hour off' light cycle, and an average surface light intensity of 84 μmol m^{-2} s^{-1}. High total inorganic nitrogen removal efficiencies (~95%) and good biomass settleability (sludge volume index 53–58 mL g^{-1}) were observed in both PSBRs. Higher biomass density was observed at higher SRT, resulting in greater light attenuation and less oxygen production. A mathematical model was developed to describe the algal–bacterial interactions, which was based on Activated Sludge Model No. 3, modified to include algal processes. Model predictions fit the experimental data well. This research also proposes an innovative holistic approach to water and energy recovery. Wastewater can be effectively treated in an anaerobic digester, generating energy from biogas, and later post-treated using an algal–bacterial PSBR, which produces biomass for additional biogas production by co-digestion.

Key words | algal–bacterial consortium, high strength wastewater treatment, light attenuation, mathematical model, photobioreactor, shortcut nitrogen removal

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INTRODUCTION

Anaerobic digestion (AD) of domestic, industrial and agricultural wastes stabilizes organic matter and produces biogas that can be used as an energy source. However, effluents from AD contain high NH_4^+-N concentrations, which can induce eutrophication in natural waters. The conventional biological nitrogen removal pathway for such effluents is the combination of nitrification and denitrification. Innovative shortcut nitrogen removal processes (i.e. nitritation–denitritation) have been developed over the past decade that save up to 25% of energy for aeration and 40% of carbon source requirements compared with conventional nitrification–denitrification processes (Wiesmann *et al.* 2006). Aeration costs could be further reduced by using algae photosynthesis for oxygen supply. Studies have shown that wastewater treatment systems containing algal–bacterial consortia may provide additional energy savings and higher nutrient removal efficiency, when compared to systems that rely only on either algal or bacterial processes (Liang *et al.* 2013). This algal–bacterial symbiosis can be applied in photobioreactors to reduce the concentrations of nutrients while reducing the electrical energy demands from aeration in wastewater treatment processes (Kouzuma & Watanabe 2015). In these reactors, the photosynthetic activity of microalgae provides oxygen needed for organic matter oxidation and nitrification during the light periods. Denitrification or denitritation processes take place primarily during the dark (anoxic) period.

The availability of light inside the photobioreactor is a major factor for microalgal photosynthesis, affecting the oxygen production process. Light availability is affected by concentrations of dissolved organic compounds and total suspended solids (TSS), which are related to the photobioreactor operating conditions, particularly the solids retention time (SRT). An SRT of 15 days was shown to result in complete nitrification without mechanical aeration in a study

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using a consortium of algae and nitrifiers to treat synthetic wastewater (50 mg NH_4^+-N L^{-1}) in photo-sequencing batch reactors (PSBRs) (Karya *et al.* 2013). Wang *et al.* (2015) treated centrate from anaerobically digested swine manure with higher ammonium concentration (300 mg NH_4^+-N L^{-1}) and also achieved complete ammonia removal via nitritation–denitritation in PSBRs with alternating light and dark periods and SRT of 8 days.

Although these authors and others (de Godos *et al.* 2014) recently studied algal–bacterial symbiosis for wastewater treatment there is still a lack of research on modelling the performance of algal–bacterial systems. Models are needed to predict, for example, growth of both microorganisms, efficiency of nutrient removal from wastewater during different seasons and in different geographic regions, or the effect of system design and operational parameters on overall system performance. One of the latest biological process models for use in wastewater treatment is the Activated Sludge Model no. 3 (ASM3), which better describes the decay processes compared to ASM1 and includes cell internal storage compounds (Henze *et al.* 2000). However, a disadvantage of ASM3 is the representation of nitrification and denitrification as single-step processes. Thus, the activities of the ammonium-oxidizing bacteria and archaea (AOB and AOA) and nitrite-oxidizing bacteria (NOB) are not properly distinguished. In order to be able to describe shortcut nitrogen removal, nitrite dynamics in wastewater treatment systems should be modelled. Some researchers therefore have proposed new versions of ASM3, extended to two-step nitrification and two-step denitrification, i.e. with nitrite as an intermediate (Iacopozzi *et al.* 2007; Kaelin *et al.* 2009). Models for bacterial growth could be combined with models for algal growth. Several researchers have suggested mathematical models to describe algal photosynthesis and growth kinetics, which can be expressed as a function of light conditions (Martínez *et al.* 1991; Cornet *et al.* 1995; Halfhide *et al.* 2015), temperature and pH (Costache *et al.* 2013), and inorganic carbon, inorganic nitrogen and inorganic phosphorus (Decostere *et al.* 2016).

This research proposes a holistic approach for wastewater treatment consisting of AD coupled with an algal–bacterial PSBR (Figure 1). AD is used for bioenergy production, through a combined heat and power system, and the high nutrient strength centrate is further treated in a PSBR. Biomass produced in the PSBR can be returned to the AD to increase biogas production by co-digestion with the main waste streams (Wang & Park 2015). This paper reports on experimental PSBR studies and the development and calibration of a mathematical model that represents the performance of the algal–bacterial PSBR under varying operating conditions. The model describes how light availability is affected by dissolved and suspended matter concentrations in the PSBR and how light attenuation influences oxygen production and nitrogen removal.

MATERIALS AND METHODS

Experimental

PSBRs

The design and operation of the bench-scale PSBRs used in this study have been described elsewhere (Wang *et al.* 2015). Briefly,

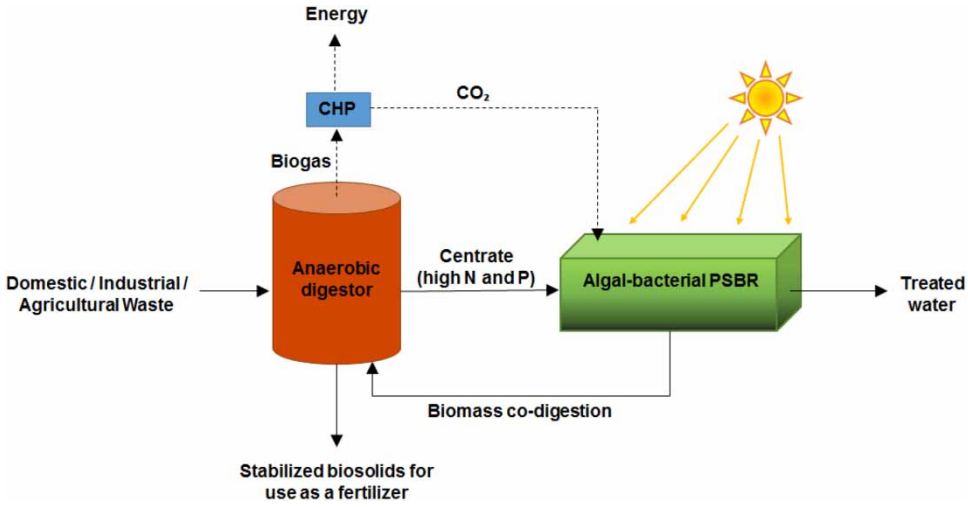

Figure 1 | Scheme of the proposed holistic approach for treatment of domestic, industrial and agricultural wastes.

two cylindrical glass reactors (2 L volume, 16 cm diameter, 10 cm height) were inoculated with a mixed microbial consortium, which contained nitrifying and heterotrophic bacteria derived from a wastewater mixed liquor seed and wild strain algae – mainly *Chlorella* spp. The PSBRs were fed with centrate from a pilot-scale mesophilic anaerobic digester that was used to process swine manure, which was collected from Twenty Four Rivers Farm (Plant City, FL, USA) on a weekly basis, mixed with urea and local groundwater and fed to the digester three times per week. Urea was added to make up for the loss of urine due to the farm operation. The swine centrate was centrifuged for 15 min at a speed of 4,000 rpm, filtered with a 0.45 μm membrane filter and diluted three times before being used to feed the PSBRs, with an average NH_4^+-N concentration of 264 ± 10 mg L^{-1}. Typical characteristics of the influent are shown in Table SM1 in the supplementary material (available with the online version of this paper).

The operation of the PSBRs consisted of a 24 hour cycle (feed, react, settle, decant), of which 12 h were illuminated and 12 h were dark. The PSBRs were continuously stirred at 200 rpm using a magnetic mixer, except during settling and withdrawal stages at the end of the dark period. The experiment was divided into two phases (Figure 2); in Phase 1 no external carbon source was applied while during Phase 2 sodium acetate was added at the start of the dark period to promote denitrification, based on the stoichiometry of 2.2 g COD g^{-1} NO_2^--N removed (COD: chemical oxygen demand). Between Phase 1 and Phase 2, a 4-day dark period was applied when sodium acetate was added (amount needed for full denitrification) to the PSBRs to eliminate accumulated NO_2^--N from Phase 1. No inflow or outflow was introduced during the 4-day dark period. No CO_2 was added during the operational steps since alkalinity was sufficient for the nitrification and algae growth (1,574 mg $CaCO_3$ L^{-1}).

The hydraulic retention time was maintained at 4 days in both PSBRs, but each reactor was operated at a different SRT. Reactor 1 (R1) was operated with an average SRT of 7 days and Reactor 2 (R2) of 11 days. SRTs were maintained by withdrawing a portion (R1: 250 mL, R2: 150 mL) of the mixed liquor each day, just before the settling period. The SRT was calculated by Equation (1):

$$\text{SRT (d)} = \frac{TSS_R\ V_R}{TSS_R\ V_W + TSS_E\ V_E} \quad (1)$$

where TSS_R is the biomass concentration of the mixed liquor (mg L^{-1}); TSS_E is the biomass concentration of the effluent (mg L^{-1}); V_R is the reactor volume (L); V_W is the daily volume of wasted mixed liquor (L d^{-1}) and V_E is the daily volume of effluent (L d^{-1}).

Incident light

The PSBRs were irradiated with two banks of eight cool white fluorescent tubes (Philips Cool White-20 W, 24 inches), placed on two sides of the reactors, providing an average light intensity on the surface of the PSBRs of 84 ± 3 μmol m^{-2} s^{-1}. Incident light intensity was measured with a Quantum MQ-200 meter (Apogee Instruments, USA) at eight different points along the reactors' wall and the light intensity considered for both PSBRs is given as the average value of these measurements.

Light attenuation

The light intensity (I) within the PSBRs cannot be merely represented by the light intensity at the surface of the PSBRs. Light attenuation causes a considerable reduction

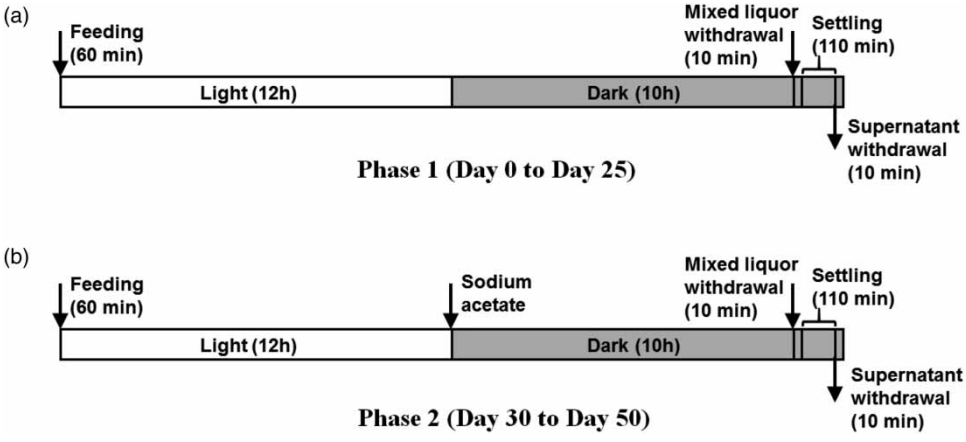

Figure 2 | Operational steps of the PSBRs during one cycle of (a) Phase 1: no sodium acetate addition and (b) Phase 2: with sodium acetate addition at the start of the dark period.

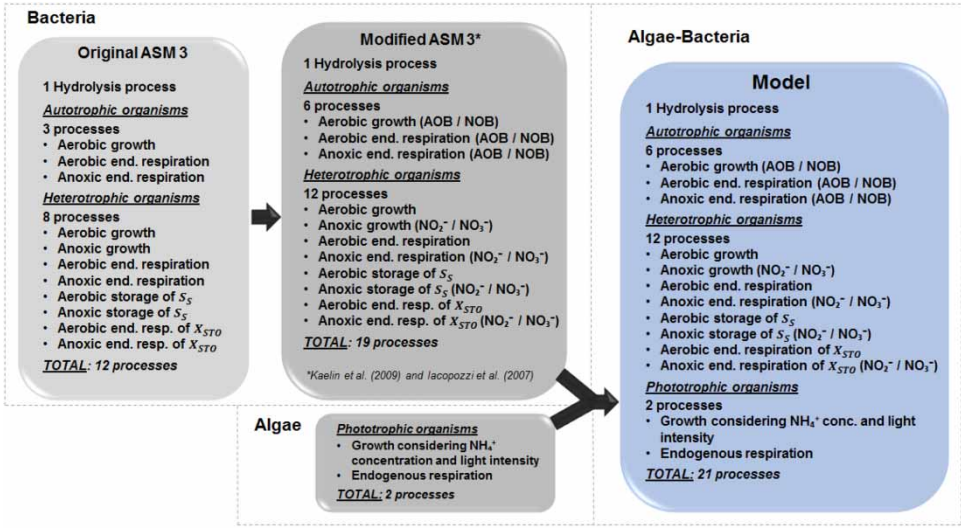

Figure 3 | Schematic of model structure elaboration, combining modified ASM3 and new algal processes to propose the algal–bacterial model.

in light intensity along the depth of the reactor. The modified Beer–Lambert law was applied to describe the light intensity at a specific position from the light source as (Martínez *et al.* 1991):

$$I(x) = I_0 \exp(-kX_T x) \quad (2)$$

where I_0 is the initial light intensity (μmol m^{-2} s^{-1}), k is the extinction coefficient (m^2 g^{-1} TSS), X_T is the TSS concentration (g TSS m^{-3}) and x is the distance from the light source (m).

The light intensity was measured at nine different points along the reactor radius (every 1 cm distance from 0 cm to 8 cm), at varying distance from the light source, inside one of the PSBRs, using a Quantum meter MQ-200 (Apogee Instruments, USA). This procedure was repeated with seven different concentrations of mixed liquor and influent to study the influence of TSS concentration on the light availability inside the PSBR. All the dilutions were made using the influent, and the first concentration (C1) corresponds to the influent without any algal–bacterial biomass. The data collected from this experiment were used to determine the extinction coefficient, k, in Equation (2) using the MS Excel tool 'Solver' (generalized reduced gradient nonlinear algorithm).

Analytical methods

The pH and dissolved oxygen (DO) were measured with an Orion GS9156 pH and DO meter (Thermo Fisher Scientific Inc., Waltham, MA, USA), respectively, and calibrated electrodes. Chlorophyll-*a* was measured using the ethanol extraction method according to NEN 6520 – Dutch Standard (NEN 2006). TSS and volatile suspended solids were measured according to standard method 2540 D (APHA 2012). The concentrations of NH_4^+, NO_2^- and NO_3^- were measured using a Metrohm Peak 850 Professional AnCat ion chromatography system (Metrohm Inc., Switzerland), with method detection limits (MDLs) of 0.20, 0.04 and 0.01 mg L^{-1}, respectively. Total nitrogen (TN) of samples was measured using Hach Total Nitrogen Reagent set TNT 828 (Hach Inc., USA).

Integrated algal–bacterial model

The mathematical model was mainly based on the parameters and rates defined by ASM3, which comprises processes of autotrophic bacteria (nitrifiers) and heterotrophic bacteria (denitrifiers). Nitrification and denitrification are represented as single-step processes in ASM3; therefore, modifications were made according to methodology proposed by Iacopozzi *et al.* (2007) and Kaelin *et al.* (2009). Nitrification was separated into two processes with NH_4^+ and NO_2^- as substrates for autotrophic bacteria, AOB and NOB respectively. Denitrification was divided into two steps with NO_3^- and NO_2^- as substrates for heterotrophic bacteria.

Since algal processes and rates are not accounted for in ASM3, two processes were incorporated, related to algal growth and endogenous algal respiration (Figure 3). Similar to the methodology described by Martínez *et al.* (1991), the algae growth was represented by an exponential model, which is one of the most common kinetic models for representing the variability of algae specific growth rate with light

intensity:

$$\mu = \mu_{\max,P}\left[1 - \exp\left(-\frac{I}{I_s}\right)\right] \quad (3)$$

where μ is the algae specific growth rate (d^{-1}), $\mu_{\max,P}$ is the maximum specific growth rate for algae (d^{-1}), I is the actual light intensity (μmol photon m^{-2} s^{-1}) and I_s is the saturation light intensity (μmol photon m^{-2} s^{-1}).

The modified Beer–Lambert law was used to incorporate the light intensity variation into the model. Considering Equation (2), it is possible to calculate the point-by-point variation in light intensity inside the PSBR. However, it is very complex to establish this variation in a cylindrical reactor, so an analogy with a parallelepiped was applied to calculate the mean light intensities (I_m) by integrating Equation (2) from $x = 0$ to $x = L$ (length of the light pathway inside the reactor) and dividing by L:

$$I_m = \frac{I_0}{kX_T L}[1 - \exp(-kX_T L)] \quad (4)$$

The average specific growth rate can be described by substituting I_m in Equation (3) and assuming that the algal cells adapt to the average value of light intensity and grow as if continuously exposed to that light intensity (Martínez *et al.* 1991). Integrating the effect of NH_4^+ substrate concentration (expressed as a Monod equation) and the average light intensity, the algal biomass growth rate is represented by r (g COD m^{-3} d^{-1}):

$$r = \mu_{\max,P}\frac{S_{NH_4}}{K_{NH_4,P} + S_{NH_4}} \left\{1 - \exp\left(\frac{-I_o[1 - \exp(-k\,X_T\,L)]}{k\,X_T\,L\,I_s}\right)\right\} X_P \quad (5)$$

where S_{NH_4} (g NH_4^+-N m^{-3}) is the NH_4^+-N concentration, $K_{NH_4,P}$ is the NH_4^+ half-saturation constant (g NH_4^+-N m^{-3}) and X_P is the phototrophic biomass concentration (g TSS m^{-3}).

The phototrophic endogenous respiration rate, R (g COD m^{-3} d^{-1}), was defined using the same type of mathematical expression as is used for endogenous respiration rates for bacteria as:

$$R = b_P\,X_P \quad (6)$$

where b_P is the endogenous respiration constant for phototrophs (d^{-1}).

The mathematical equations were set into the software AQUASIM 2.0 (Reichert *et al.* 1995) to perform simulations, calibration and sensitivity analysis. The model calibration was done using the data collected hourly during one cycle (24 hours) of R1 on day 49, Phase 2. The initial conditions used as input for the calibration are shown in Table SM2 of the supplementary material (available with the online version of this paper). The Aquasim tool 'Sensitivity analysis' was used in order to identify the most sensitive parameters. Afterwards, the calibration was done using the tool 'Parameter estimation' to estimate new values for the most sensitive parameters, based on the profiles of NH_4^+-N, NO_2^--N, NO_3^--N and DO. The methodology for the sensitivity analysis and calibration is described by Reichert *et al.* (1995).

Statistical analysis

A statistical analysis applying the *t*-test (two tailed paired) was performed to compare the hourly NH_4^+ removal and NO_2^- formation rates between R1 and R2 during the light period of one cycle. Data from three cycles (Days 14, 42 and 49) were recorded and the average values were used for the statistical analysis. The NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the effluent of R1 and R2 during Phase 1 and Phase 2 were analysed by single-factor analysis of variance (ANOVA) ($\alpha = 0.05$) using Minitab 16 (PA, USA). The root-mean-square error (RMSE) was used to calculate the error between the values for R1 measured during the experimental period and the values predicted by the model.

RESULTS AND DISCUSSION

An algal–bacterial consortium was successfully cultured in two PSBRs for 50 days. The biomass developed good settleability with a sludge volume index of 53 mL g^{-1} for R1 and 58 mL g^{-1} for R2. In addition, steady nitritation–denitritation was observed with TN removal over 90% achieved (see below). Measurements of nitrogen species, biomass concentration and light attenuation were combined with operational parameters to obtain data to calibrate the model.

Experimental

PSBRs

Average effluent NH_4^+-N and NO_2^--N concentrations were significantly higher (single-factor ANOVA, $p < 0.05$) in Phase 1 than in Phase 2 for both PSBRs (Table 1). Total inorganic

Table 1 | Average NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the influent and effluent of R1 (SRT 7 d) and R2 (SRT 11 d). Effluent NH_4^+-N and NO_2^--N concentrations were significantly different between phases, for both reactors

	Influent		Effluent					
	R1 and R2		R1			R2		
	Phase 1	Phase 2	Phase 1	Phase 2	*p* value*	Phase 1	Phase 2	*p* value*
NH_4^+-N (mg L^{-1})	290 ± 3	236 ± 19	83 ± 9	1 ± 1	5.14×10^{-12}	106 ± 8	5 ± 2	2.73×10^{-14}
NO_2^--N (mg L^{-1})	5 ± 0	3 ± 0	97 ± 11	24 ± 7	8.52×10^{-8}	70 ± 10	16 ± 3	1.26×10^{-5}
NO_3^--N (mg L^{-1})	< MDL	1 ± 0	2 ± 1	< MDL	–	1 ± 0	< MDL	–

Differences between reactors were not significant (single factor ANOVA 95% confidence interval).
*p value of ANOVA between Phase 1 and Phase 2.

nitrogen (TIN) removal efficiencies during Phase 1 were approximately 38% and 40% for R1 and R2, respectively. NO_2^- removal by denitrification was most probably hindered by the lack of a readily biodegradable carbon source remaining until the dark period. Likewise, Kinyua *et al.* (2014) reported that, compared to the total COD of anaerobically digested swine manure, the readily biodegradable COD fraction was very low (4–5%). Wang *et al.* (2015) showed that little denitritation occurred without addition of an external carbon source when treating anaerobically digested swine manure in a PSBR. Furthermore, previous studies of systems treating wastewater with high levels of total NH_4^+-N and free ammonia have reported inhibition of NOB activity (Vadivelu *et al.* 2007; Kouba *et al.* 2014), favouring partial nitrification (i.e. nitritation). Consequently, NO_2^- accumulation was observed in the PSBRs during Phase 1 (Figure 4). For this reason, sodium acetate was added to the PSBRs and a 4-day full dark period was implemented to provide conditions required for denitritation. During Phase 2, sodium acetate was added just before the dark cycle to ensure enough readily degradable carbon source for NO_2^- reduction, enhancing TIN removal efficiencies for R1 and R2 to 95% and 94%, respectively.

Light attenuation measurements

This study allowed a better understanding of the light attenuation inside the PSBRs and further analysis of how the light attenuation, TSS concentration, oxygen production and nitrogen removal are interlinked. Light intensity varied with distance from the light source inside the reactor and was affected by TSS concentrations (C1 to C7) (Figure 5). By fitting Equation (2) to these results, the light coefficient k was determined as 0.0748 ± 0.0048 m^2 g^{-1} TSS, later used as an input for the model calibration.

A further analysis based on the light intensities along the light path inside the PSBR at varying TSS concentrations was performed to approximately calculate and compare the portion of irradiated and completely dark volumes in each of the PSBRs. TSS concentrations C5 (1,480 mg TSS/L) and C6 (2,167 mg TSS/L) were the ones closest to the average in R1 (1,357 ± 58 mg TSS/L) and R2 (1,744 ± 88 mg TSS/L), respectively. The completely dark volumes were assumed to be the radial portion from the point in which there was no light detected by the quantum meter. For example, for C5 the light intensity was zero from 6 cm to 8 cm while for C6 the light intensity was zero from 4 cm to 8 cm distance (Figure 6).

These values indicate that a higher algal–bacterial biomass concentration hindered the photosynthetic activity in R2 due to the shading effect of the TSS. Therefore not all biomass was continuously irradiated. The average total biomass during the experiment in R2 was 1.44 times higher than in R1. However, applying the percentage of irradiated volume (Figure 6) for both reactors and considering only the irradiated biomass, the ratio is almost equalized, lowering the value from 1.44 to 1.09. This indicates that the amount of irradiated algal biomass in both reactors was very similar. Although it is a rough estimation, one can assume that since the oxygen production in the algae chloroplasts is directly related to the light availability, the gross oxygen production for both reactors was similar. The net oxygen production by algae is the gross production minus the oxygen used for algal endogenous respiration. The latter increases with the biomass concentration, and therefore the net oxygen production is probably lower in R2 than in R1. As a result there is more oxygen available for AOB in R1 than in R2 and indeed the NH_4^+ removal and NO_2^- formation rates were significantly higher for R1 than for R2 ($p < 0.05$) (Figure 7). In addition, the average biomass productivity during the experiment was 187 ± 8 mg L^{-1} in R1 and 156 ± 9 mg L^{-1} in R2. The DO profiles, which are discussed below, also confirmed that more oxygen was

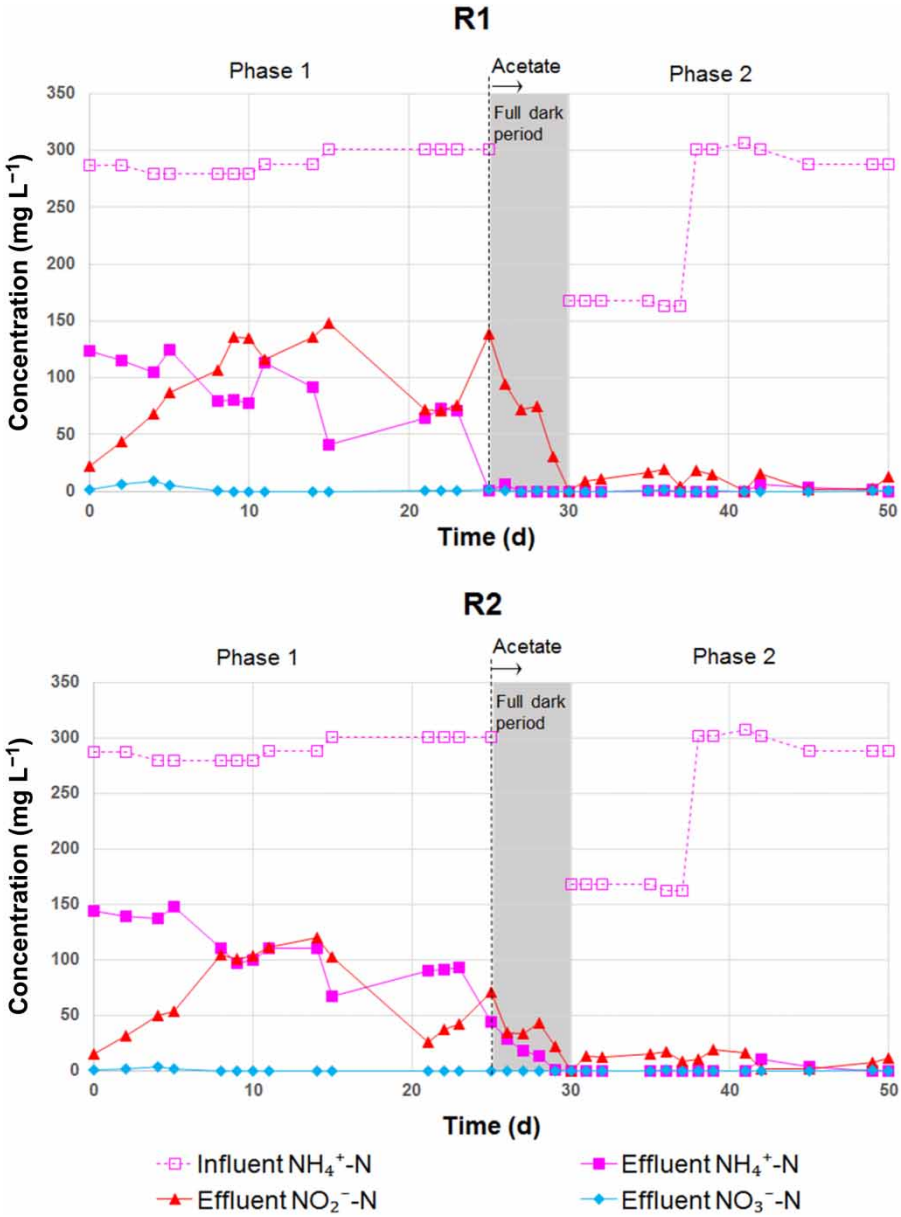

Figure 4 | Influent and effluent ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) concentrations over time in R1 (SRT 7 d) and R2 (SRT 11 d).

available in R1, since the increase in DO towards the end of the light period was higher and started earlier. These results and comparisons indicate that higher SRT resulted in higher TSS concentrations in R2, decreasing the light intensity and oxygen availability for AOB inside the PSBR.

As shown in Figure 6, R1 had a higher estimated irradiated volume, due to a lower TSS concentration. It is important to note that these estimations are based on radial decrease of light intensity, while the actual light distribution inside the PSBRs was probably similar to an elliptical shape. This is an artefact of the experimental set-up, in which the light was applied from the sides of the PSBRs. In a full-scale algal pond, light would be coming from the surface of the pond.

In photobioreactors with only algal biomass, productivity is maximized when the light intensity is above the compensation light intensity at all locations inside the photobioreactor. Under such conditions all the algal cells are photosynthesizing and there is no dark zone, which increases the biomass productivity (de Mooij *et al.* 2016). Based on the observed light attenuation in R1, this would require an SRT that is lower than 7 days, to allow further

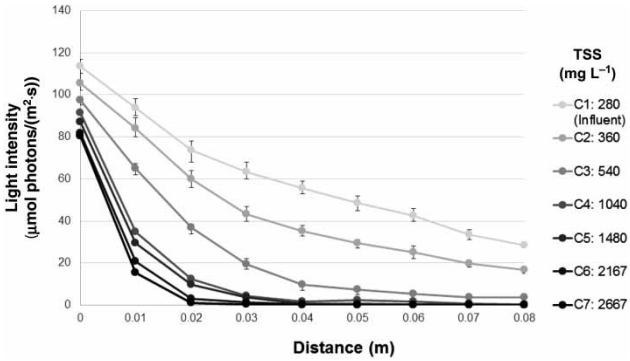

Figure 5 | Light intensities measured at varying distance from light source inside the PSBR, and varying TSS concentrations (C1–C7).

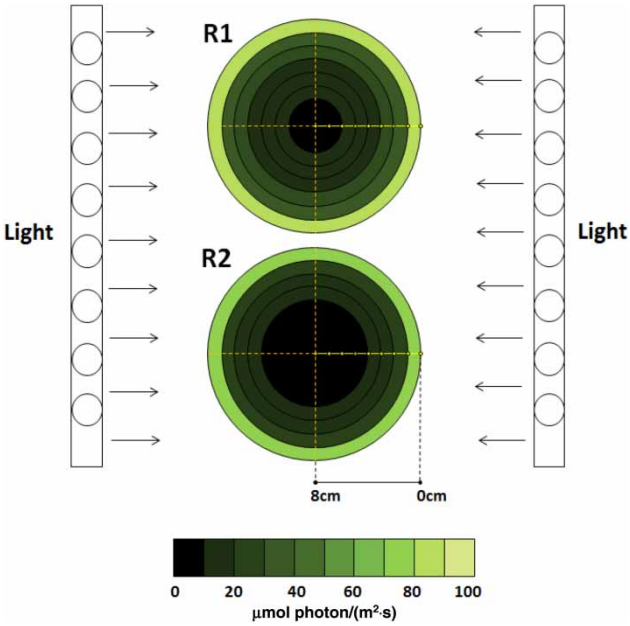

Figure 6 | Estimation of irradiated zones at varying light intensities (R1: 98%, R2: 75% of reactor volume), and completely dark zones (R1: 2%, R2: 25% of reactor volume) inside both PSBRs.

light penetration inside the reactor. Rada-Ariza *et al.* (2015) observed NH_4^+ removal from 77–96 mg NH_4^+-N L^{-1} in the influent to less than 4 mg NH_4^+-N L^{-1} in the effluent, when the SRT was 3 days or larger. When the SRT was shortened to 1 day, the effluent concentration increased to 18 mg NH_4^+-N L^{-1}. This shows that if the SRT in algal–bacterial systems is too low, slow-growing AOB are washed out of the reactor. Therefore, an optimum SRT should be slightly above the minimum SRT for nitrifiers in order not to decrease the light availability more than necessary. However, as AOB are sensitive to light, the dark zone may also have a secondary benefit as it could protect these microorganisms from photoinhibition (Yoshioka & Saijo 1984). Furthermore, the presence of a dark zone likely prompted simultaneous nitritation–denitritation during the light period, which was also reported by Wang *et al.* (2015). This indicates the presence of aerobic and anoxic zones inside the PSBRs in addition to the most probable existence of DO gradients within the algal–bacterial flocs.

In summary, the experiments showed that SRT and light intensity are important factors affecting nutrient removal efficiency in PSBRs, and that SRT should be chosen to optimally balance growth requirements of algae and AOB, since they are combined in one single system.

Integrated algal–bacterial model

The list of variables and parameters used in the model, the list of processes and rates and the stoichiometric matrix are provided in the supplementary material (available with the online version of this paper). Profiles of measured values and model predictions of nitrogen species and DO for the light period in both reactors showed a good fit to the experimental data (Figure 7). The results for the sensitivity analysis indicated the maximum specific growth rate of phototrophs ($\mu_{max,P}$), saturation constant of NH_4^+ for phototrophs ($K_{NH_4,P}$) and saturation light (I_s) as the most sensitive coefficients for the predictions of nitrogen species and DO. Hence, the calibration resulted in adjusted values for these coefficients (see Table SM3, available online). The following RMSE values were calculated: 8.0 (NH_4^+-N), 6.8 (NO_2^--N), 0.5 (NO_3^--N) and 1.4 (DO). However, the predicted NH_4^+ release during the dark period was significantly higher than observed. An assumption of the model is that only algal 'endogenous respiration' takes place in the absence of light, as well as the bacterial processes. The effect of those processes on NH_4^+ release should be considered. Decostere *et al.* (2016) proposed a microalgal growth model, which includes respiration and an additional decay process; however, both these processes do not affect the NH_4^+ concentration. In contrast, the heterotrophic respiration taken from ASM3 includes NH_4^+ release (see Table SM5, available online). Apparently the mixed algal–bacterial biomass releases much less NH_4^+ than is observed for endogenously respiring bacteria. This may be explained by the fact that decay and cell disruption are lumped together in ASM3 as 'endogenous respiration'. And decay and disintegration of bacterial cells may occur at higher rates than algal cell disintegration, due to the strong algal cell wall. Therefore, further studies related to the decay and disintegration of algae biomass could elucidate the absence of NH_4^+ release during the dark period (Edmundson & Huesemann 2015).

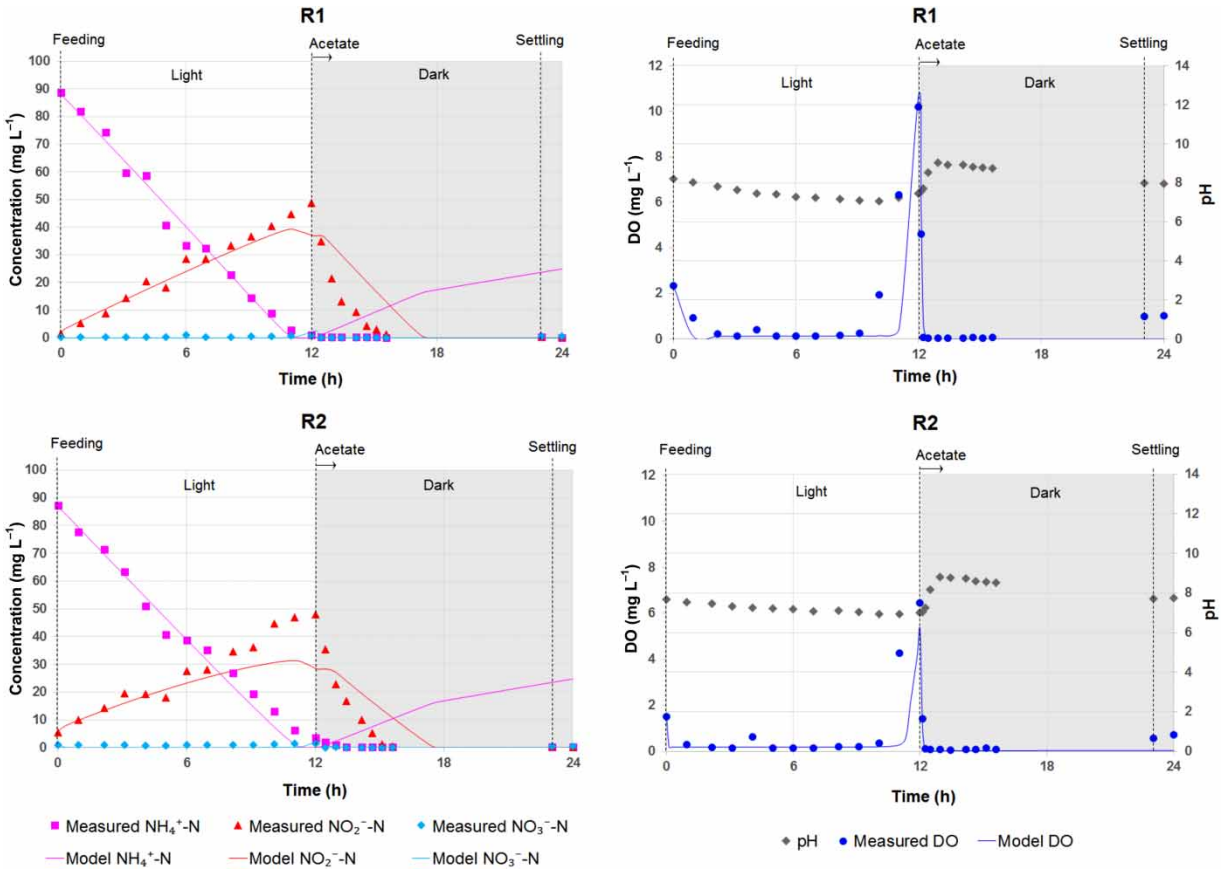

Figure 7 | Profiles of model predictions and experimental data of nitrogen species and DO for both reactors, during one cycle (Phase 2, Day 49).

The predicted formation and removal of NO_2^- followed the same trend as the experimental data, although the observed decrease in NO_2^- was faster than predicted by the model. This could have been because of an underestimation of the growth rate of denitrifiers, considering that the influent and internally generated COD were ignored. NO_3^- concentrations remained low (<3 mg L^{-1}) throughout the experiments due to the shortcut process of nitritation–denitritation in both experimental data and model performance.

In order to compare the performance of NH_4^+ removal in algal–bacterial and algal-only systems, the model was used to simulate PSBR performance with an assumption that R1 contained only algal biomass. This simulation was done using the uncalibrated model (i.e. with parameters from the literature; see Table SM3) and inactivating the bacterial processes in the software (Figure 8).

As expected, the simulation did not fit to the observed values, since the AOB and NOB activities were not included. However, the results indicate that NH_4^+ uptake solely by algae in a PSBR occurs at a slower rate than for a mixed consortium of microalgae and nitrifying bacteria (Rada-Ariza *et al.* 2017). Therefore, the NH_4^+ removal simulated is much lower than the measured values in the PSBRs with the algal–bacterial consortium. The proportion of algae and bacteria in the biomass in R1 was approximately calculated based on the stoichiometry and dry weight obtained from the experiment. The algal–bacterial biomass composition was estimated to be 67% algae, 16% heterotrophs and 17% nitrifiers. The percentage for nitrifiers was similar to that observed in a study carried out by van der Steen *et al.* (2015). The stoichiometric oxidation of NH_4^+ by microbial conversion (nitrification) is much higher compared to the uptake from algal growth. This explains why, even if the algal biomass concentration was much higher than the bacterial biomass concentration, the AOB activity plays an important role in the decrease of NH_4^+ concentration in simulations for combined systems. Hence, when assuming only algal biomass, the NH_4^+ removal is considerably lower than in algal–bacterial systems. In this experiment, the NH_4^+-N removal during one cycle (Phase 2, day 49) was 177 mg NH_4^+-N in R1, from which 96 mg NH_4^+-N (54%) was removed by nitritation–denitritation, and 174 mg NH_4^+-N in R2, from which 87 mg NH_4^+-N (50%) was removed by

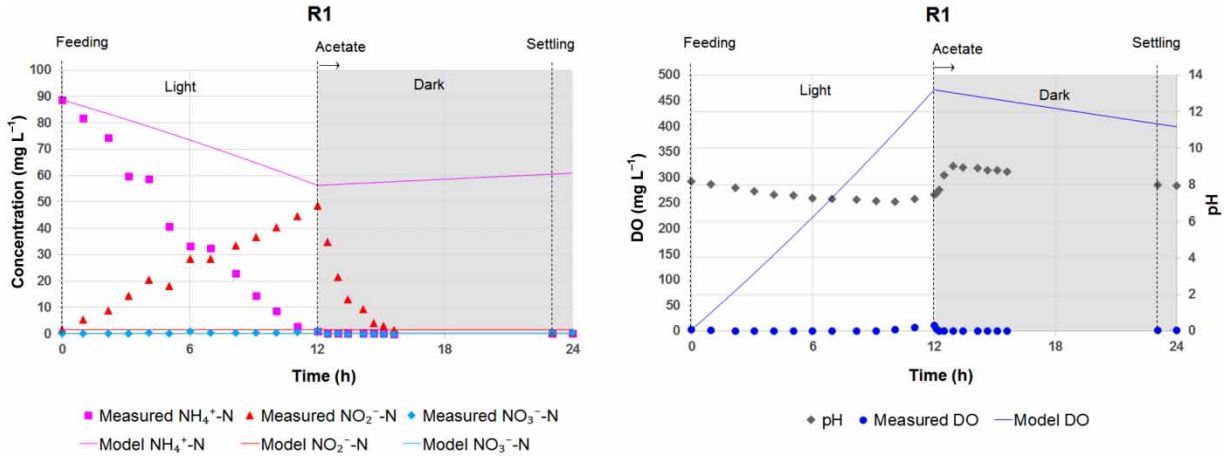

Figure 8 | Simulations of the base model (uncalibrated) considering an algal system in R1, i.e. with no bacterial processes incorporated.

nitritation–denitritation. Karya *et al.* (2013) and Wang *et al.* (2015) reported higher values, with approximately 85% of NH_4^+-N removal in algal–bacterial systems, which was due to nitrification, and only 15% by algae uptake. It is important to note that the algal performance in this model was only based on NH_4^+ concentration and light availability, but other factors may also be important (i.e. phosphorus concentration, alkalinity and pH).

The model presented in this paper, therefore, can help to evaluate nitrogen removal dynamics, as well as to predict the most relevant operating conditions that accelerate or restrict processes in algal–bacterial systems.

CONCLUSIONS

The proposed holistic process has the potential to recover bioenergy from domestic, industrial and agricultural waste while producing treated effluents that can be reused or safely discharged to receiving waters without causing eutrophication. TIN removal (95%) from high NH_4^+ strength wastewater (264 mg NH_4^+-N L^{-1}) using an algal–bacterial consortium in PSBRs was successfully achieved by nitritation–denitritation processes, provided that a biodegradable carbon source was supplied. The operational control of SRT had an important effect on the NH_4^+ removal in the algal–bacterial systems. An SRT of 11 days led to higher TSS concentrations than at SRT of 7 days, hindering the light availability for microalgae due to the self-shading by algal and microbial cells. Consequently, less net oxygen production was observed, decreasing the nitritation rates.

The model developed provided satisfactory results, although further improvements are needed to describe the effect of endogenous respiration on NH_4^+ concentrations during the dark periods of the PSBR cycle. This tool can be useful to design and optimize the operations of PSBRs for different applications (e.g. maximizing algal productivity, minimizing effluent TN concentration) and different geographic locations and seasons.

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