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Algal-bacterial shortcut nitrogen removal model with seasonal light variations

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ABSTRACT

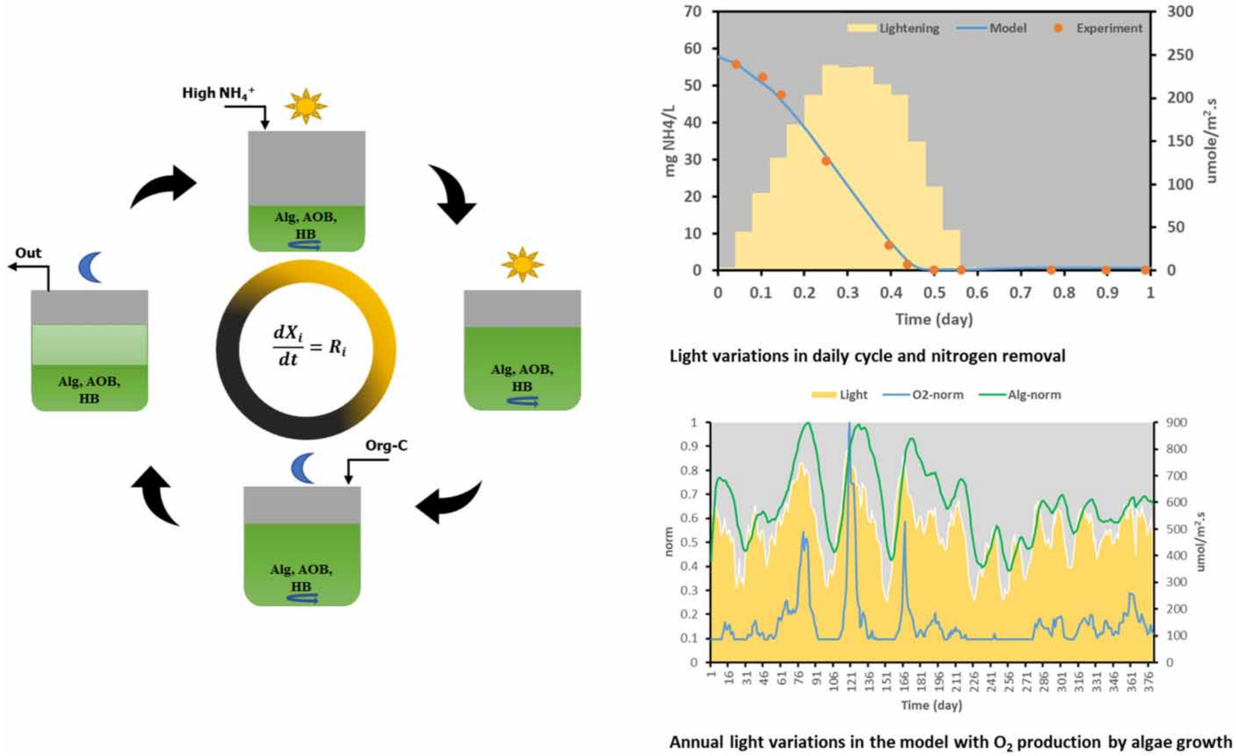
The algal–bacterial shortcut nitrogen removal (ABSNR) process can be used to treat high ammonia strength wastewaters without external aeration. However, prior algal–bacterial SNR studies have been conducted under fixed light/dark periods that were not representative of natural light conditions. In this study, laboratory-scale photo-sequencing batch reactors (PSBRs) were used to treat anaerobic digester sidestream under varying light intensities that mimicked summer and winter conditions in Tampa, FL, USA. A dynamic mathematical model was developed for the ABSNR process, which was calibrated and validated using data sets from the laboratory PSBRs. The model elucidated the dynamics of algal and bacterial biomass growth under natural illumination conditions as well as transformation processes for nitrogen species, oxygen, organic and inorganic carbon. A full-scale PSBR with a 1.2 m depth, a 6-day hydraulic retention time (HRT) and a 10-day solids retention time (SRT) was simulated for treatment of anaerobic digester sidestream. The full-scale PSBR could achieve >90% ammonia removal, significantly reducing the nitrogen load to the mainstream wastewater treatment plant (WWTP). The dynamic simulation showed that ABSNR process can help wastewater treatment facilities meet stringent nitrogen removal standards with low energy inputs.

Key words: full-scale design, hydraulic retention time, mathematical model, photo-sequencing batch reactor, shortcut nitrogen removal, sidestream wastewater

HIGHLIGHTS

- Sidestream wastewater treatment by algal–bacterial shortcut nitrogen removal.
- Varying illumination simulated summer/winter outdoor conditions.
- Model developed for dynamic PSBR simulation under varying illumination.
- Long-term simulation employed for TN removal in a full-scale WWTP.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Anaerobic digestion (AD) sidestreams contain high ammonium (NH_4^+) concentrations and have low organic carbon to nitrogen (C/N) ratios. These wastewaters are often recycled back to mainstream wastewater treatment processes, making it difficult for facilities to meet stringent nutrient discharge limits. AD sidestreams are costly to treat using conventional biological nitrogen removal (BNR) processes due to their high oxygen (O_2) demands for nitrification and lack of sufficient organic carbon for denitrification. The shortcut nitrogen removal (SNR) process has been developed to reduce O_2 and organic carbon requirements by suppressing the growth of nitrite-oxidizing bacteria (NOB) that transform nitrite (NO_2^-) to nitrate (NO_3^-). NOB suppression is accomplished by maintaining high free ammonia (FA), high free nitrous acid (FNA) and/or low dissolved oxygen (DO) concentrations in the bioreactor (Anthonisen *et al.* 1976; Wang *et al.* 2015). The use of SNR results in a 25% reduction in aeration requirements for NO_2^- to NO_3^- oxidation and a 40% reduction in organic electron donor requirements for NO_3^- to N_2 reduction and reduced sludge production compared with conventional BNR (Peng & Zhu 2006).

Integrating an algal–bacterial consortium into the SNR process can further reduce the need for external aeration, as algae can provide O_2 needed for aerobic heterotrophic bacteria (HB) and ammonia-oxidizing bacteria (AOB) (Wang *et al.* 2018). Wang *et al.* (2015) demonstrated that an algal–bacterial shortcut nitrogen removal (ABSNR) process could remove NH_4^+ from AD sidestream with no external aeration other than mixing. The authors carried out the ABSNR process in a bench-scale photo-sequencing batch reactor (PSBR) with alternating light and dark periods (12 h light/12 h dark). During the light period, O_2 was produced by algal photosynthesis, providing aerobic conditions for nitritation. During the dark period, the system became anoxic, providing favorable conditions for denitritation. Improved biomass production and nitritation efficiency was observed at higher light intensities. Arun *et al.* (2019) investigated the ABSNR process in a PSBR for the treatment of wastewater with $C/N < 0.5$ using a consortium of algae, AOB and methanol-consuming HB. Li *et al.* (2021) proposed a symbiotic algae-based SNR technology to reduce the cost of high NH_4^+ strength wastewater treatment. The authors used biofilm colonization in the algal–bacterial PSBR and studied the impacts of hydraulic retention time (HRT), aeration rate and carbon source addition on nitrogen removal efficiency.

All of the aforementioned ABSNR studies were carried out under artificial lighting with fixed light intensities during the light period (Wang *et al.* 2015, 2018; Arun *et al.* 2019; Li *et al.* 2021). However, full-scale photobioreactors, such as high-rate algal ponds (HRAPs), are usually operated under outdoor conditions, with day and night light cycles following seasonal patterns. During the summer, the high light intensity can increase DO concentrations to levels that may be toxic to algae and AOB (Nishi *et al.* 2020). While low light intensity during the winter or under overcast conditions will decrease photosynthetic O_2 production and may require shallower reactors, supplemental illumination and/or longer HRT to maintain AOB growth. Operation of the PSBR at shallow depth or long HRT increases system footprint, land area requirements and capital costs. In addition, reduced nitrogen loading rates can reduce NOB suppression due to low FA concentrations (Duan *et al.* 2020). These challenges should be considered in PSBR design according to the influent wastewater composition and seasonal changes in light intensity. Therefore, mathematical models are needed to predict the efficiency of the ABSNR process under varying illumination similar to outdoor conditions and to address full-scale design challenges.

Several prior modeling studies have investigated algal–bacterial interactions and nitrogen removal pathways in ABSNR systems operated under fixed light intensities and dark periods (typically 12 h light/12 h dark). Arashiro *et al.* (2017) developed a mathematical model of the ABSNR process. The model was calibrated using data from a laboratory-scale ABSNR system operated with AD sidestream under varying operating conditions. The model described how total biomass concentration and solids retention time (SRT) affected light attenuation in the PSBR and consequently DO production and nitrogen removal. Peng *et al.* (2018) proposed a modeling framework for algal growth using a photo-inhibition parameter instead of mean light intensity. Synergistic and competitive growth kinetics were used to simulate algal–bacterial interactions in their study. Arun *et al.* (2019) also examined algal–bacterial interactions through metabolic models developed to predict nitrogen removal mechanisms based on algae-AOB and algae-AOB-HB activities. In a prior study by our group (Shayan *et al.* 2022), an ABSNR model was developed to predict the contributions of various nitrogen removal processes and the dominance of various biomass species during daily and long-term operating conditions (e.g., SRT, organic carbon requirements). The model was able to estimate the performance of laboratory-scale PSBRs fed with an AD sidestream under fixed light and dark periods with constant light intensity.

Solimeno & García (2019) developed the BIO-ALGAE model, which incorporated varying light conditions. The model simulated the dynamics of algae and bacteria in an HRAP over a year at different HRTs. The authors showed that the HRAP had different treatment capacities with changing seasons but did not propose a design to accommodate seasonal variations for annual operations. In addition, the BIO-ALGAE model did not include the NOB suppression required to simulate the SNR process for treatment of a high NH_4^+ strength wastewater.

The aim of this study was to improve our previously developed model (Shayan *et al.* 2022) to include dynamics of algae, AOB, NOB and HB and dissolved chemical species (e.g., DO, organic and inorganic C, NH_4^+, NO_2^-, NO_3^-) in ABSNRs operated with varying light intensities expected under outdoor operating conditions. The model was calibrated and validated using data from laboratory-scale PSBRs operated under light conditions mimicking winter (January 1990) and summer (June 1990) conditions in the Tampa Bay area (FL, USA), allowing us to test the performance under highly varying conditions. The model was used to investigate the impact of varying HRTs on algal–bacterial growth. Nitrogen removal performance was simulated for a full-scale PSBR used to treat AD sidestream wastewater in the Tampa Bay Area over an entire year using daily illumination data from 2020.

2. MATERIALS AND METHODS

2.1. Laboratory-scale PSBR design and operation

Details of the laboratory-scale PSBR design can be found in Shayan *et al.* (2022). Briefly, duplicate laboratory-scale PSBRs (2 L working volume) were operated in the following five stages (Figure 1): (1) feeding AD sidestream, (2) mixing under light conditions (nitritation), (3) addition of an organic carbon source (sodium acetate) and mixing under dark conditions (denitritation), (4) settling, and (5) decanting. The algal–bacterial consortium used was initially collected from an algal mat in a clarifier at a local wastewater treatment plant (WWTP) and was used in a prior study on the treatment of anaerobically digested swine waste sidestream (Wang *et al.* 2015). Genera and species of the algae from the PSBR were identified by Zongo *et al.* (2023), who showed that the algal biomass consisted primarily of *Chlorella* spp. (30% of the total biomass), *Scotiellopsis* sp. (25%), *Zynemopsis* sp. (25%) and *Actinastrum* sp. (15%). Biomass was wasted daily to

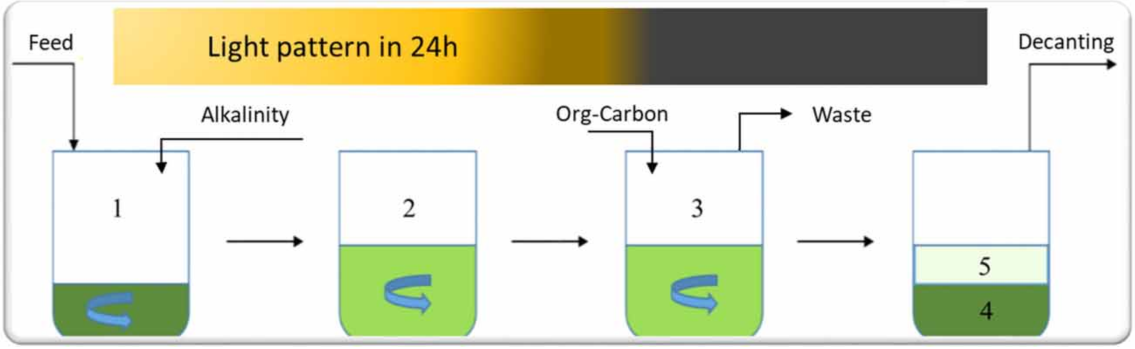


Figure 1 | Photo-sequencing batch reactor (PSBR) schematic with details on operating cycles under varying illumination.

maintain the design SRT and the wasting volume was calculated using the following equation (Wang *et al.* 2015):

$$\mathrm{SRT} = \frac{\mathrm{TSS_R} \times V_\mathrm{R}}{(\mathrm{TSS_R} \times Q_\mathrm{w}) + (\mathrm{TSS_e} \times Q_\mathrm{e})} \quad (1)$$

where TSS_R and TSS_e represent total suspended solids (TSS) concentrations in the reactor and in the effluent (mg/L); V_R is the reactor volume (L); Q_w is the daily volume of mixed liquor wasted (L/day) during stage 3, and Q_e is daily volume of the effluent discharged (L/day) after settling in stage 5. In addition, a backup algal–bacterial consortium was maintained the in refrigerator without aeration or CO_2 supplementation.

Alkalinity was adjusted at the beginning of each light period by adding $MgCO_3$ to maintain the ratio of 3.4 mg $CaCO_3$/mg NH_4^+-N for nitritation. Because the Mg^{2+} concentrations varied in the semi-synthetic centrate, the initial Mg^{2+} concentration varied from 65 to 150 mg/L. According to Daneshgar *et al.* (2018), $MgCO_3$ addition can improve settling by facilitating the reaction between Mg^{2+}, PO_4^{3-} and NH_4^+ to produce struvite, which precipitates in the pH range of 7–11. After nitritation in the light period and NO_2^- generation, sodium acetate was added at the beginning of each dark period to initiate denitritation based on a stoichiometric molar ratio of acetate to NO_2^- as 0.975:1.

2.2. Varying light intensity setup for laboratory-scale PSBR

Outdoor daily light irradiance data were obtained from a meteorological station at Tampa International Airport (FL, USA). Typical average daily light patterns were selected for summer (June 1990) and winter (January 1990). The light was provided to the laboratory-scale PSBRs at programed intensities with eight steps using poly-chromatic LED lights (KIND LED K5 Series XL750 Indoor Grow Light, USA) located above the reactor. Outdoor summer and winter light intensities were used to determine the equivalent mean light intensities for the laboratory-scale PSBR (Figure S1) using an exponential model (Martinez Sancho 1991). These values were adjusted for self-shading by biomass using the Beer–Lambert law (Equation (2a)) and approximate biomass concentration and depth of the reactor.

$$I_\mathrm{m} = \frac{I(1 - \exp(-kXL))}{kXL} \quad (2a)$$

where I_m is the mean light intensity in laboratory-scale or full-scale PSBR (μmol/m^2 s), I is the meteorological station light intensity or the light incidence generated by the LED lamps (μmol/m^2 s), X is the total biomass density in the laboratory-scale or full-scale PSBR (mg TSS/L) which is the sum of AOB (X_{AOB}), NOB (X_{NOB}), HB (X_{HB}), algae (X_P), inert particulate organics (X_I) and slowly biodegradable particulate substrate (X_S), k is the total biomass extinction coefficient (m^2/g), L is the depth of the reactor (0.08 m for laboratory-scale). Note that the total biomass extinction coefficient (0.07 m^2/g) used was from a previous study by our team using a similar PSBR and wastewater (Arashiro *et al.* 2017).

A depth of 0.5 m and a total biomass concentration of 2,500 mg/L were assumed to calculate I_m for each hour of a day in a full-scale PSBR. The I_m in a full-scale PSBR should be equal to the mean light intensity in the laboratory-scale system with a depth of 0.08 m and the same biomass concentration (Equation (2b)).

$$\frac{I_{fs}(1-\exp(-kXL_{fs}))}{kXL_{fs}} = \frac{I_{ls}(1-\exp(-kXL_{ls}))}{kXL_{ls}} \quad (2b)$$

where fs and ls represent full-scale and laboratory-scale PSBRs, respectively.

The Monod equation was used for algal growth, considering growth was limited by mean light intensity, NH_4^+ and inorganic carbon (CO_2 and HCO_3^-) availability (Equation (3)).

$$\mu = \mu_{max,alg}\left(\frac{NH_4^+}{NH_4^+ + k_{s,NH_4}}\right)\left(\frac{CO_2 + HCO_3^-}{k_{s,C} + CO_2 + HCO_3^-}\right)\left(1-\exp\left(-\frac{I_m}{I_s}\right)\right) \quad (3)$$

where μ is the specific growth rate and $\mu_{max,alg}$ is the maximum specific growth rate of algae (day^{-1}), k_{s,NH_4} is the saturation coefficient for NH_4^+-N (mg/L), $k_{s,C}$ is the saturation coefficient for inorganic carbon (mg/L), I_s is the light saturation intensity (μmol/m^2 s). Note that the temperature was not considered as a factor in this equation due to the sub-tropical climate in Tampa, FL. In addition, it was assumed that the PSBR would be treating sidestream from a mesophilic AD (35–37 °C). However, this parameter should not be neglected for simulations in cold regions with higher HRTs.

A complete description of the process kinetic and equilibrium equations is provided in section 2.5 and Table S1 of the supplementary information. In addition to algae, nitrifying bacteria and heterotrophic growth were modeled using Monod equations. NH_4^+-N, DO and alkalinity concentrations were limiting factors for AOB growth. For NOB growth, NO_2^--N and DO were limiting factors and NH_4^+-N was an inhibiting factor. Aerobic growth of HB was limited by readily biodegradable chemical oxygen demand (rbCOD), DO and NH_4^+-N and anoxic growth of HB included rbCOD, NO_2^--N as limiting factors and DO as an inhibiting factor (Shayan *et al.* 2022). The following equilibrium equations (Equations (4) and (5)) were used at the beginning and the end of the light period to evaluate SNR conditions and NOB inhibition by FA and FNA (Ford *et al.* 1980):

$$\text{FA (mg/L)} = \frac{17}{14}\cdot\frac{[NH_4^+ - N]\times 10^{pH}}{10^{pH} + \exp\left(\frac{6,344}{273+T}\right)} \quad (4)$$

$$\text{FNA (mg/L)} = \frac{46}{14}\cdot\frac{[NO_2^- - N]}{10^{pH}\times\exp\left(\frac{-2,300}{273+T}\right)} \quad (5)$$

2.3. Wastewater feed composition and operating conditions

A semi-synthetic AD sidestream was prepared using screened raw wastewater collected from the Falkenburg Advanced Wastewater Treatment Plant in Hillsborough County, Florida, with added NH_4Cl, K_2HPO_4 and $MgCO_3$ to achieve target concentrations summarized in Table 1. The semi-synthetic wastewater was formulated to mimic the characteristics of real sidestream from our prior studies, which was obtained by centrifuging biomass from a pilot AD-treating waste-activated sludge (Zalivina 2019). As mentioned previously, sodium acetate was added as the electron donor at the beginning of the dark period to facilitate denitritation. Summer mode was studied in Phase 1 at an HRT of 4 days, resulting in a nitrogen loading rate (NLR) of 87.5 mg N L^{-1} day^{-1}. Winter mode was studied in Phase 2 at an HRT of 8 days, resulting in an NLR of 43.75 mg N L^{-1} day^{-1}.

2.4. Analytical methods

Grab samples were normally collected twice per week. Additional samples for model calibration and validation were collected during hourly sampling campaigns performed over a full cycle (24 h). Nitrogen species and phosphate concentrations were measured using a Metrohm Peak 850 Professional An/Cat-ion chromatography (IC) system (Metrohm Inc., Switzerland). DO and pH were measured *in situ* using calibrated Orion GS9156 meters (Thermo Fisher Scientific Inc., Waltham, MA, USA). Chlorophyll α was analyzed via NEN 6520-Dutch Standard. Samples were filtered through a 0.45 μm filter for soluble COD measurements using Lovibond COD test kits (Tintometer Inc., USA). rbCOD was measured using a

Table 1 | Operating parameters and average feed composition, for laboratory PSBRs

Operating parameters	
Working volume	2 L
Hydraulic retention time (HRT)	4-day (summer), 8-day (winter)
Solids retention time (SRT)	10-day
Feed volume added per cycle	500 mL (summer), 250 mL (winter)
Light intensity	Daily pattern (Figure S1)
Operating pH range	6.5–8.5
Operating alkalinity range	400–700 mg $CaCO_3$/L
Feed composition	
NH_4^+-N	350 ± 10 mg/L
NO_2^--N	< 4 mg/L
NO_3^--N	BDL
PO_4^{3-}-P	60 ± 5 mg/L

BDL, below detection limit.

rapid physical–chemical method (Mamais *et al.* 1993). Alkalinity was measured as $CaCO_3$ using Standard Method 2320B. Total biomass density was measured as TSS using Standard Methods 2450B. Light intensity was measured by an ExTech Easyview 30 light meter (ExTech Inc., Waltham, MA, USA) at the liquid surface of the reactor.

2.5. Model description and reactor design

The model used the same structure as the algal–bacterial PSBR model presented in our prior study (Shayan *et al.* 2022), which combined parameters and process equations from Activated Sludge Model No.1 (ASM1), River Water Quality Model No.1 (RWQM1) and BIO-ALGAE (Henze *et al.* 2000; Reichert *et al.* 2001; Solimeno *et al.* 2017a; Solimeno & García 2019). The model includes 17 dissolved and particulate species and 24 physical, chemical, and biochemical process rates (Table S1). Furthermore, a matrix of stoichiometric coefficients and their values were listed in Tables S2 and S3 in the supplementary information. Table S4 also included the biokinetic, chemical and physical parameters in the model. No biological processes were considered during the last 2 h of settling and decanting. Effluent characteristics from each cycle were used as influent values for the next cycle. Since the PSBRs were operated under LED lights that mimicked outdoor seasonal light patterns, the model was modified to use light intensity as a function of time. In addition, the sensitive kinetic parameters listed in the prior study were re-evaluated and calibrated manually within the ranges in the literature to minimize the errors between the model predictions and the experimental results according to changes in operating conditions. The calibrated model was validated using data from hourly sampling campaigns carried out on PSBRs operated in summer and winter modes.

The validated model was used to simulate a full-scale PSBR assumed to be used for the treatment of AD sidestream generated at the South Cross Bayou (SCB) Water Reclamation Facility in Pinellas County, Florida. The case study was suggested in our preliminary investigation (Shayan 2021), with typical AD sidestream conditions for SCB reported by Medina (2020) and summarized in Table 2. Ten-day moving averages of daily light irradiances for the year 2020 from a nearby monitoring station were used. Global solar irradiance is usually measured in W/m^2 and an approximate conversion factor of 4.6 is typically used to estimate light intensity in μmol/m^2 s (Carruthers *et al.* 2001). However, since ~45% of solar radiation falls in the photosynthetic active range, the conversion factor used in this study was 1 W/m^2 = 2.1 μmol/m^2 s.

3. RESULTS AND DISCUSSION

3.1. Laboratory-scale PSBR operation

Laboratory-scale PSBRs were operated under summer and winter illumination conditions using the converted light intensities and influent wastewater concentrations listed in Table 1. Under each condition (summer/winter) the PSBRs were operated for at least 30 daily cycles after a steady state was reached. Weekly samples from specific times (start, mid, end) were taken to calculate means and standard deviations (Tables 3 and 4).

Table 2 | Influent wastewater characteristics in the full-scale model (Medina 2020)

Parameter	Influent wastewater
Flow rate	557 m^3/day
NH_4^+-N	715 mg/L
NO_2^--N	1 mg/L
NO_3^--N	0.1 mg/L
HB (X_H)	150 mgCOD/L
AOB (X_{AOB})	50 mgCOD/L
NOB (X_{NOB})	1 mgCOD/L
Alg (X_P)	2 mgCOD/L
Inert particulate organic matter (X_I)	20 mgCOD/L
Slowly biodegradable organic matter (X_S)	20 mgCOD/L
Readily biodegradable substrate (S_S)	50 mgCOD/L
pH	7.5
Alkalinity	850 $mgCaCO_3$/L

Table 3 | Mean values and standard deviations (SD) of oxygen and nitrogen species from weekly samples at specific times under summer conditions

	0.04		0.25		0.9	
Time (day)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
O_2 (mg/L)	0.11	0.05	0.35	0.04	0.45	0.19
NH_4^+-N (mg/L)	56.33	2.25	30.33	1.75	0.14	0.23
NO_2-N (mg/L)	0.07	0.08	5.75	0.44	2.85	0.22
NO_3^-N (mg/L)	0.03	0.05	0.02	0.04	0.005	0.008

Table 4 | Mean values and standard deviations (SD) of oxygen and nitrogen species from weekly samples at specific times under winter conditions

	0.02		0.25		0.9	
Time (day)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
O_2 (mg/L)	0.10	0.05	0.43	0.05	0.005	0.008
NH_4^+-N (mg/L)	55.31	3.21	27.24	1.86	0.21	0.23
NO_2-N (mg/L)	9.34	0.98	22.26	2.06	4.67	0.42
NO_3-N (mg/L)	1.12	0.44	1.61	0.08	1.02	0.075

Nitrogen species and DO concentrations for summer mode were analyzed based on samples collected hourly over 24 h on day 75. Results from the 24 h study showed nitritation and denitritation during day and night periods (Figure 2). Also, a simulation based on kinetic parameters from our previously developed model for constant light intensity (Shayan *et al.* 2022) and using initial conditions from current experiments, was conducted. Deviations of the model simulations from the experimental results were observed (Figure 2); therefore, several adjustments were implemented on relative proportions of microbial biomass in the reactor to capture dissolved species trends. For example, modeled HB concentrations were decreased, and algae concentrations were increased to provide more O_2 available for AOB. The experimental results showed that NH_4^+-N was already converted to NO_2^--N at the beginning of the cycle, indicating algae can grow under low light intensity and AOB was able to consume any produced O_2 at the beginning of the cycle leading to anoxic conditions.

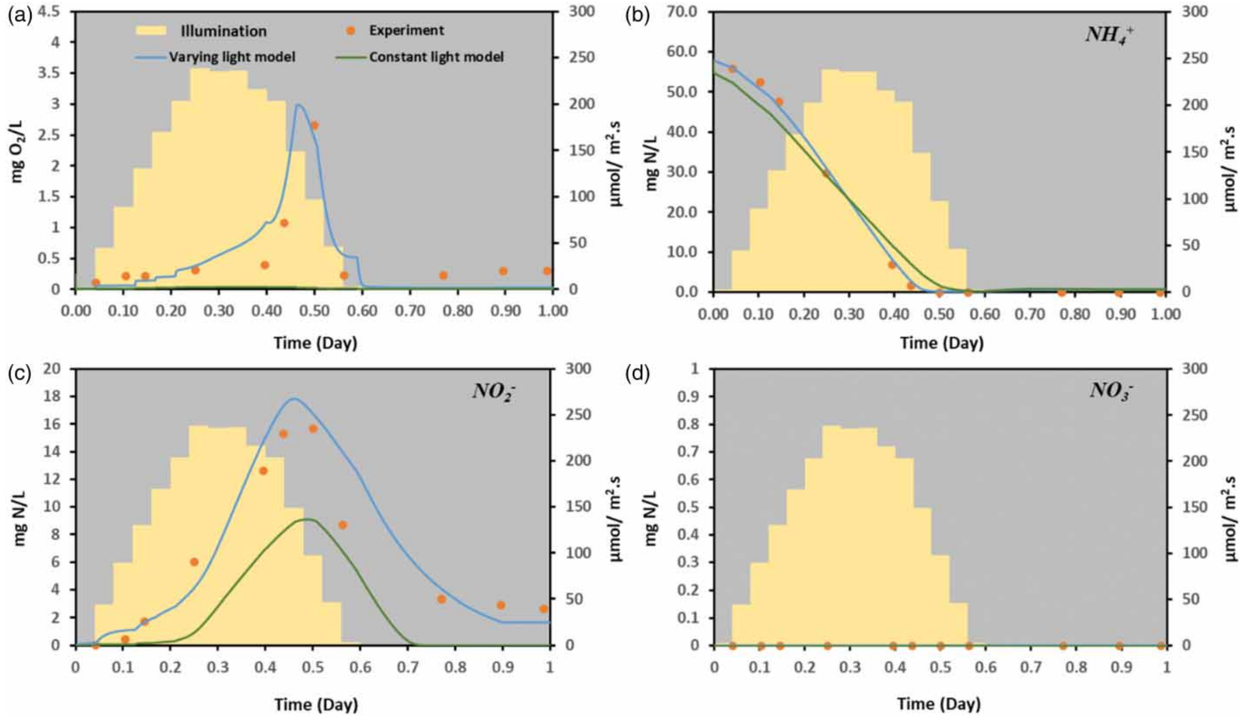


Figure 2 | Dynamic simulation of 24 h summer mode operation with 4-day HRT and using the kinetic parameters obtained for constant light/dark mode (green line) and recalibrated parameters for the varying light condition (blue line).

The adaptations of the biomass to outdoor light conditions and resulting changes in nitrogen removal efficiency justified the recalibration of the model for varying light intensity conditions. Table 5 shows the sensitive parameters and adjusted values compared with values obtained for the constant light intensity study. Simulation results using the recalibrated kinetic parameters are shown in Figure 2. Sensitivity analysis was carried out by manually varying the kinetic parameters ($\pm 10\%$) and checking the target concentration response. This was the same method used in a previous study under constant illumination (Shayan 2021). As an example, Figure S2 in the supplementary information shows O_2 variations with different b_H values (0.35–0.55 day^{-1}). Sensitive parameters were adjusted and calibrated manually and individually based on experimental conditions and reported values or ranges in similar studies (Table S4 in supplementary information). The primary reason for

Table 5 | Sensitive parameters for model based on gradual light illumination and their values for both constant and variable light illumination

Kinetic parameters	Constant light illumination (Shayan *et al.* 2022)	Variable light illumination	Unit
b_H	0.35	0.5	day^{-1}
b_{alg}	0.2	0.1	day^{-1}
K_{I,NH_4}	65.5	7.5	mg/L
$K_{NH_4,AOB}$	0.8	1.7	mg/L
$K_{NH_4,alg}$	1.5	0.5	mg/L
$K_{NO_2,H}$	0.5	6.5	mg/L
$K_{NO_3,H}$	3	1	mg/L
$K_{O_2,AOB}$	0.028	0.1	mg/L
$K_{O_2,H}$	0.85	0.8	mg/L
$K_{O_2,NOB}$	0.3	0.81	mg/L
$K_{S,H,L}$	5	20	mg/L

some significant changes is switching the PSBR operation from constant light into varying light intensities. Since biomass growth, nitrogen removal and oxygen generation were adapted for new operating conditions under long-term operation, some kinetic parameters needed to be significantly adjusted. For example, the ammonium inhibition constant, K_{I,NH_4}, was decreased from 65.5 to 7.5 mg/L to reflect greater NOB inhibition under varying light intensity and get closer to the value reported by Solimeno *et al.* (2017b) for an outdoor HRAP. The half saturation constant of HB for NO_3^- ($K_{NO_3,H}$) was decreased to account for NO_3^- consumption by HB during the light period and simultaneous nitritation/denitritation. The half saturation constant of HB for NO_2^- ($K_{NO_2,H}$) was increased to describe NO_2^- accumulation under varying light intensity without rapid denitritation. Furthermore, to reflect low HB growth at early light periods, the saturation constant for rbCOD ($K_{S,H,L}$) was increased significantly to minimize HB growth in early light period and competition with AOB. This value was also reported by Solimeno *et al.* (2017b) for outdoor HRAP operations. The half saturation constant for algae growth on NH_4^+ ($K_{NH_4,alg}$) was decreased to reflect the algae growth under low NH_4^+ concentrations when there is higher light intensity in daytime. This can promote photosynthesis, algae growth and O_2 generation by increased illumination. On the other hand, $K_{O_2,AOB}$ and $K_{NH_4,AOB}$ were increased to reflect the lower AOB growth rate during low O_2 availability periods at the beginning of the cycle compared with the constant light/dark model (Shayan *et al.* 2022).

The recalibrated model predictions generally matched well with the measured concentrations of the dissolved species, as shown in Figure 2. The model was able to simulate O_2 consumption during $0 < t < 0.1$ day due to AOB and HB growth, and O_2 accumulation after declining NH_4^+ concentration and decreasing AOB growth (Figure 2(a)). Nitrogen species diagrams showed NH_4^+ oxidation and NO_2^- production during the daytime (Figure 2(b)) and denitritation during the night (Figure 2(c)). Figure 2(d) showed that a negligible amount of NO_3^- was produced over the complete cycle, which confirmed the SNR process. Increases in O_2 concentrations at the end of the cycle ($0.9 < t < 1$ day) were most likely due to disturbances of the PSBR by sampling the biomass during the settling phase. In addition to R^2, a comparison of the root mean square error (RMSE) and normalized RMSE (NRMSE) between the simulation results from constant and varying light kinetic parameters, showed the importance of recalibration for studies under varying light intensities (Table 6).

A conversion rate diagram for O_2 was constructed for summer light intensity with HRT of 4 days (Figure 3) to simulate the gradual production of O_2 by increasing the light intensity and the O_2 consumption by the AOB growth, HB growth and HB respiration. The O_2 consumption; however, is dominated by AOB growth leading to O_2 depletion if the kinetic parameters for the constant light/dark model are used (Figure S3 in Supplementary material). Another O_2-consuming process was aerobic respiration of algae, which had a negligible rate of -10 mg/L day at the end of the light period. Similar to our prior study (Shayan *et al.* 2022), the model was able to simulate rbCOD conversions during nitritation and denitritation to verify the cause of DO accumulation (Figure S4). Anoxic conditions occurred during $0 < t < 0.1$ day due to low DO availability and equal O_2 consumption and production rates. Thus, NO_2^-/NO_3^- were utilized as electron acceptors during this time frame. In addition, negligible NO_3^- concentration showed NOB suppression and the SNR process was successful in this trial.

Model validation was carried out using experimental data from a second 24 h study conducted under summer illumination conditions and a 24 h study conducted under winter illumination. Validation results for summer mode for DO and nitrogen species are illustrated in Figure 4. The primary reason for the different nitrogen and oxygen patterns in Figures 2 and 4 was different initial NH_4^+-N concentrations in the semi-synthetic sidestream. As shown in Figure 4(a) and 4(b), a higher NH_4^+-N concentration increased oxygen demand, decreasing O_2 availability and limiting nitritation. Equal O_2 production and consumption rates during the daytime provided anoxic conditions and resulted in low nitritation rates, which are shown in Figure 4(b) and 4(c). Any produced NO_2^- during the daytime was utilized as an electron acceptor for anoxic growth or respiration, which is why NO_2^--N remained < 1 mg/L. Figure 4(c) clarifies that simultaneous nitrification/denitrification (SND)

Table 6 | Comparison of R^2, root mean square error (RMSE) and normalized RMSE (NRMSE) PSBR simulations using kinetic parameters from constant light and recalibrated parameters for varying light

	O_2		NH_4^+-N		NO_2^--N		NO_3^--N	
Illumination	**Varying**	**Constant**	**Varying**	**Constant**	**Varying**	**Constant**	**Varying**	**Constant**
R^2	0.81	0.03	0.98	0.98	0.86	0.76	0.98	0.002
root mean square error (RSME) (mg/L)	0.38	0.88	1	3.37	1.37	2.69	0.001	0.001
normalized root mean square error (NRSME)	0.71	1.62	0.06	0.19	0.22	0.42	0.05	0.73

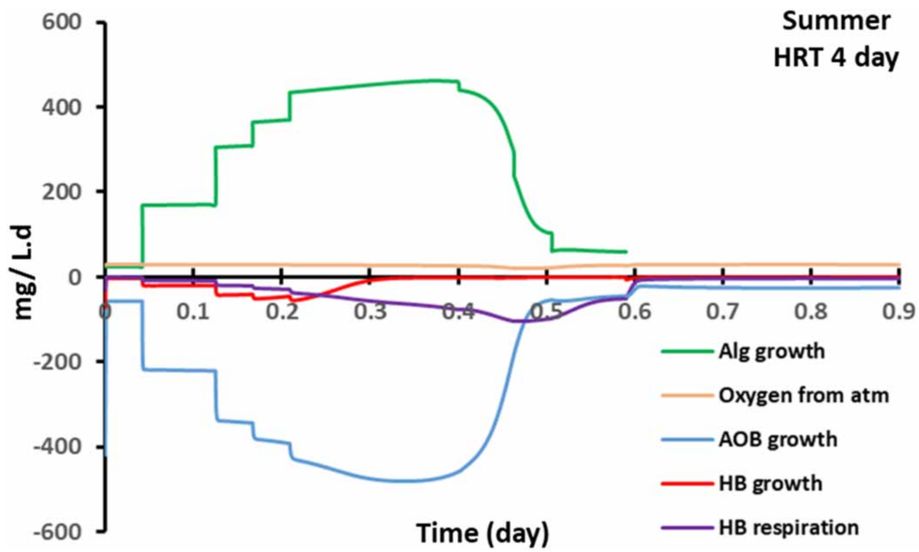


Figure 3 | Conversion rate of O_2 in a complete cycle of PSBR in summer illumination model with HRT 4 days.

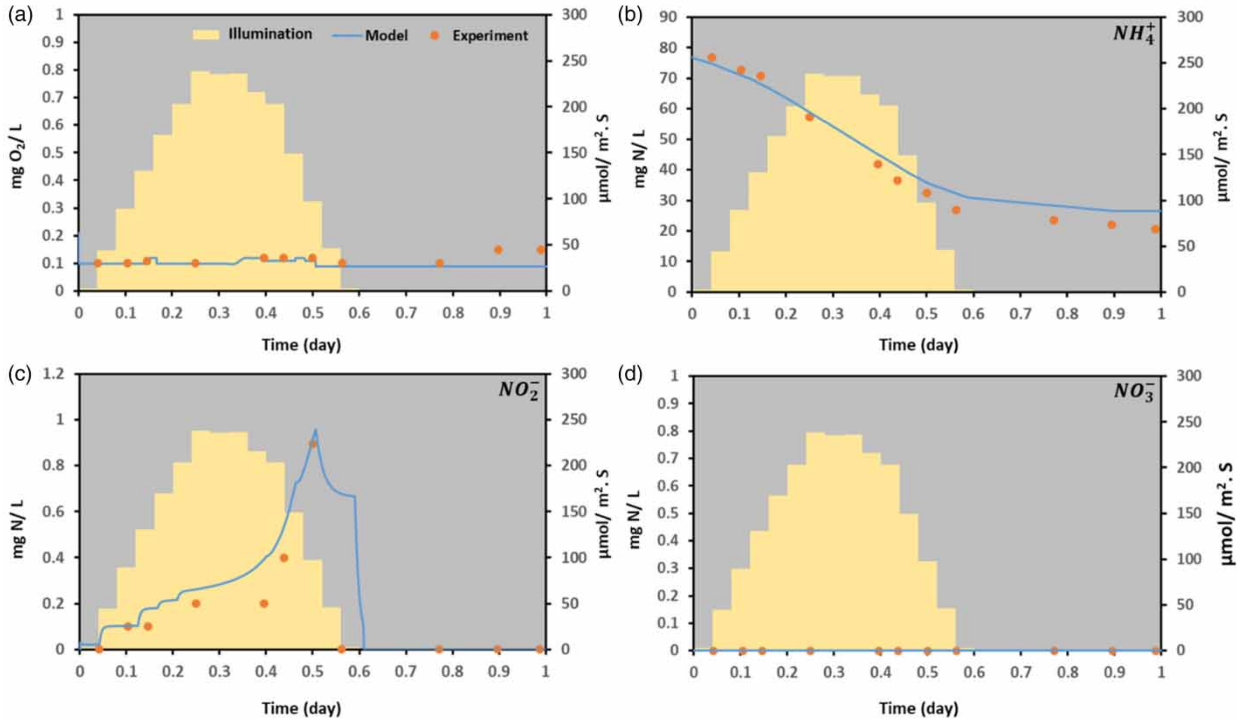


Figure 4 | Experimental results and model validation for a complete cycle of PSBR trial 2 under the summer light illumination and HRT of 4 days.

was a predominant N removal pathway by showing the trends of O_2 and NO_2^- concentration in a complete cycle. Negligible values of NO_3^- in Figure 4(d) supported the assumption of NOB suppression. Table 7 also describes the statistical analysis and errors during model validation in summer mode illumination.

PSBR performance under winter illumination conditions was analyzed based on a 24 h study on day 60 of the 120 days operating period (Figure 5). The model was employed to simulate nitrogen removal and O_2 production under winter light

Table 7 | Goodness of fit and statistical errors from model validation by summer light illumination and HRT of 4 days

	O_2	NH_4^+-N	NO_2^--N	NO_3^--N
R^2	0.83	0.98	0.79	0.98
RMSE (mg/L)	0.04	3.63	0.09	0.001
NRMSE	0.33	0.08	0.49	0.09

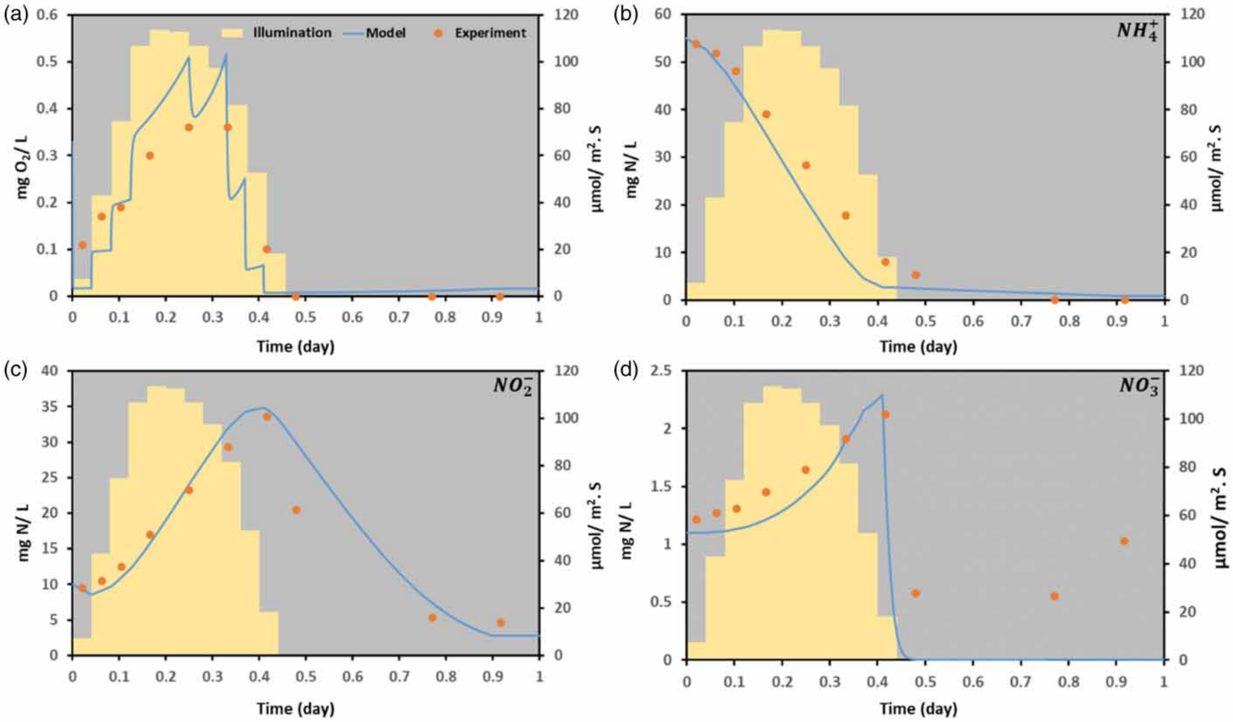


Figure 5 | Experimental results and model validation for a complete cycle of PSBR under the winter light illumination and prolonged HRT (8 days).

intensity (<120 μmol/m^2 s) with a higher HRT (8 days) to prevent biomass washout from the system. Results from DO measurements and simulation (Figure 5(a)) showed O_2 concentration increased up to 0.4 mg/L under winter conditions by gradually increasing the illumination. Successful NH_4^+ removal (Figure 5(b)) and NO_2^- production (Figure 5(c)) were achieved by algal and AOB growth during the light period. The low NO_3^- concentration (Figure 5(d)) in comparison with other nitrogen species indicated successful SNR. However, compared with summer operation, a small amount of NO_3^- production was observed, most likely because of incomplete denitrification and NO_3^- accumulation from prior cycle. Table 8 shows the statistical errors of model validation by winter light illumination with HRT of 8 days.

Table 8 | Goodness of fit and statistical errors from model validation by winter light illumination and prolonged HRT (8 days)

	O_2	NH_4^+-N	NO_2^--N	NO_3^--N
R^2	0.78	0.96	0.91	0.81
RMSE (mg/L)	0.09	3.87	3.41	0.51
NRMSE	0.57	0.15	0.21	0.38

3.2. Impacts of HRT on PSBR operation

To investigate the impact of HRT on biomass species distribution at a constant SRT of 10 days, the model was used to simulate long-term PSBR operation under summer and winter illumination conditions at HRTs of 2, 4, 6 and 8 days (Table 9). Simulation results for 120 days of PSBR operation after the acclimation phase under summer and winter conditions showed the effect of HRT on different biomass species in the model. All microbial species concentrations reached steady state within 30–50 days of simulation. The steady state and simulated concentrations of HB, AOB and NOB decreased with increasing HRT in both summer and winter modes. Longer HRT will decrease substrate loading rate (e.g., ammonia), resulting in lower biomass growth and therefore lower biomass concentrations if constant SRT is maintained.

For HB, the simulated concentrations under steady-state winter mode were higher than that in summer mode. This could be the result of lower light intensity, lower availability of O_2 and more favorable conditions for anoxic growth of HB in winter than summer. For AOB and NOB, the steady state concentrations were generally higher for summer mode compared with winter mode. This is because AOB and NOB growth are highly dependent on O_2 availability. Higher light intensities in summer mode than in winter mode resulted in greater photosynthesis rates and oxygen generation by algae growth. For algae, the impact of HRT in winter mode was different from that in summer mode. Winter mode simulations for algae showed the minimum steady state concentration at an HRT of 2 days. This could be due to higher biomass density of HB, AOB and NOB in winter mode with an HRT of 2 days, which reduces light availability in the medium and decreases algae growth.

A shorter HRT can lead to higher AOB concentrations as discussed previously; however, higher ammonia loading rates can result in high FA concentrations that can reach the FA inhibition threshold for AOB (10–150 mg/L) (Soliman & Eldyasti 2016) at the beginning of the cycle. The calculated FA concentration for an HRT of 4 days based on the NH_4^+/NH_3 equilibrium showed that FA was in the acceptable range (0.1–3.5 mg/L) to inhibit NOB growth. Also, the calculated FNA concentration for an HRT > 4 days was ~0.005 mg/L. Therefore, a longer HRT will favor NOB suppression; however, it will require a larger reactor, which may be costly. Since algae and AOB growth showed almost the same trends during HRTs of 4, 6 and 8 days, an HRT of 6 days was selected for year-round illumination conditions for a full-scale PSBR design considering the tradeoff between promoting NOB suppression and minimizing reactor volume.

3.3. Large-scale PSBR simulation

Sidestream characteristics from the SCB treatment plant in Pinellas County, FL were based on the modeling results from Medina (2020) using BioWin software (EnviroSim, Ontario, Canada). Effluent characteristics from dewatering simulations and the plant's available reports (Table 2) were used for the large-scale PSBR, assuming ideal mixing conditions. The assumed SRT and HRT for the simulations were 10 and 6 days, respectively.

Table 9 | Steady state concentration of biomass species during 120 days of PSBR simulation in summer and winter illuminations with different HRTs

	HRT 2 days	HRT 4 days	HRT 6 days	HRT 8 days
	Algae (mg/L)			
Summer	2,200	1,550	1,500	1,500
Winter	450	510	510	510
	Heterotrophic bacteria (mg/L)			
Summer	160	120	115	110
Winter	250	225	200	165
	AOB (mg/L)			
Summer	45	20	12	8
Winter	25	18	16	10
	NOB (mg/L)			
Summer	0.45	0.35	0.3	0.25
Winter	0.55	0.3	0.2	0.15

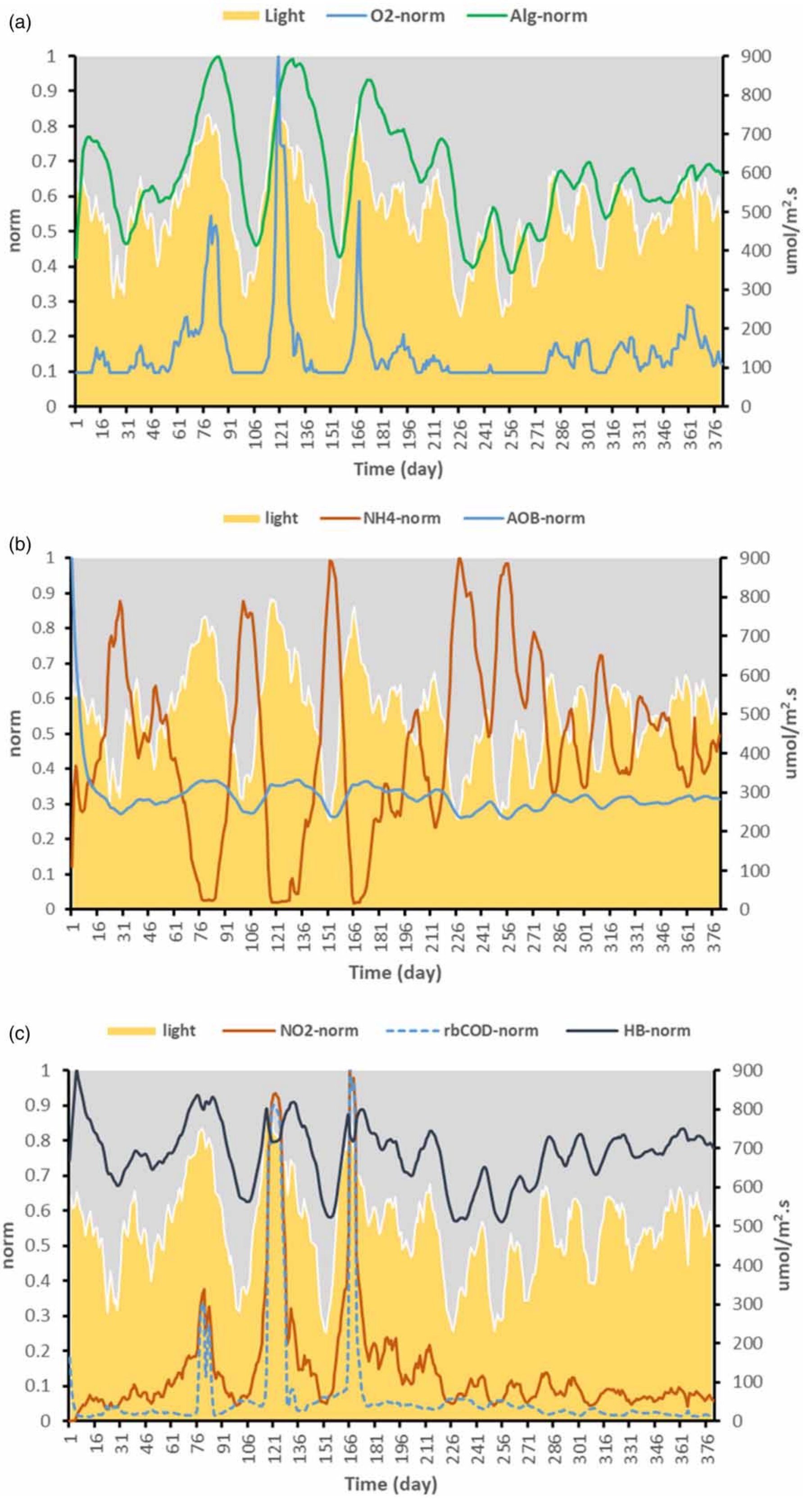


Figure 6 | Normalized concentrations of dissolved and particulate species during long-term operation of an outdoor assumed large-scale PSBR under HRT of 6 days.

Normalized biomass, DO and nitrogen species concentrations for the long-term simulation starting from 1 January are shown in Figure 6. Concentrations of dissolved and particulate species were normalized according to their maximum values as: 1,450 mg/L for algae, 121 mg/L for AOB, 346 mg/L for HB, 41 mg/L for rbCOD, 89 mg/L for NH_4^+-N, 13.5 mg/L for NO_2^--N, and 2.39 mg/L for O_2. Daily average light intensities are also shown to illustrate the impact of light intensity on microbial growth and chemical transformations. The figures describe the following trends in the simulation: (a) algae growth with O_2 production and NH_4^+ consumption; (b) nitritation with NH_4^+ consumption; and (c) denitritation with rbCOD and NO_2^- consumption. As expected, fluctuations in light intensity similarly affected algae growth and O_2 production, as shown in Figure 6(a). Low light conditions decreased NH_4^+-N removal by AOB due to low algal O_2 production, as shown in Figure 6(b). Denitritation simulated trends are shown in Figure 6(c); NO_2^--N reduction depended on rbCOD availability; however, once HB concentrations became insufficient, both NO_2^--N and rbCOD accumulated as observed on days 80, 125 and 166. It was found that HB's respiration decreased the population and unused rbCOD accumulated on these days according to the HB conversion rate diagram (Figure S5).

Fluctuations in some chemical species concentrations during long-term simulation resulted from seasonal light intensity variations (e.g., cloudy conditions) (Figure 6(a)). For example, effluent NH_4^+-N concentration of the assumed PSBR with a depth of 1.2 m, varied between 0 and 88 mg/L for a simulation period of 380 days, while the mean concentration was $<$ 50 mg/L. The dynamics of O_2 and nitrogen species concentrations in a full-scale simulation for two selected days (May 15 and November 15) were predicted and showed that nitrogen removal is higher during the summer than in winter due to greater light intensities and longer illumination periods in a complete cycle (see Figure S6 in supplementary information). In addition, pH variations were simulated for the selected days of the above-mentioned reactor and trends were similar to experimental results on a laboratory scale for summer and winter modes (Figure S7).

While ABSNR simulation for the assumed PSBR showed sufficient nitrogen removal under seasonal light variations, decreasing the reactor depth can increase light penetration to minimize concentration fluctuations over the year. Decreased reactor depth will likely result in increased land requirements and capital costs.

3.4. Recommendations for future research

The model developed in this study can be used as a tool to estimate algal–bacterial interactions in an ABSNR process under seasonal changes in light intensity. A number of assumptions were made to simplify the mathematical model, particularly with regard to the full-scale system simulation. First, we assumed ideal mixing such that dissolved and particulate matter were homogenously distributed inside the reactor. Non-ideal hydrodynamics in a real photobioreactor, such as HRAPs, results in the development of dead zones (i.e., where anoxic processes can occur), short circuiting, and affect mass transfer rate coefficients and fluxes of gases (e.g., O_2, FA) to the atmosphere (Hadiyanto *et al.* 2013). Second, we assumed a constant temperature of 20 °C; however, the temperature is known to fluctuate in outdoor algal ponds that can impact algal growth and productivity (Vindel & Trincado 2021). Third, the assumed PSBR depth was $>$ 0.5 m, which is uncommon in real designs and only used to estimate the nitrogen removal capacity for a deep reactor with limited surface area. In practice, a range of 0.1–0.3 m has been recommended for this purpose (Hadiyanto *et al.* 2013). Future studies should consider shallower ABSNR designs, which would allow for greater illumination of the algal biomass. Fourth, our model only included assimilation, nitrification, denitrification and FA volatilization as major N transformation processes; however, the formation of nitrous oxide (N_2O) as a byproduct of bacterial and algal metabolism should not be neglected (Zhang *et al.* 2022). Future studies should investigate N_2O generation in ABSNR processes. Lastly, our model assumed that sodium acetate was the carbon source for denitrification based on our laboratory-scale PSBR studies. Future studies should consider other denitritation electron donors, such as methanol, ethanol or primary sludge fermentate, and the effects of these chemicals on biodegradation rates.

4. CONCLUSIONS

In this study, a mathematical model was developed to simulate the ABSNR process under seasonal light irradiance conditions. Data from laboratory-scale PSBR studies were used for model calibration and validation. The dynamics of microbial biomass, DO, rbCOD and dissolved nitrogen species concentrations were examined over 380 days of PSBR simulation with seasonal light variations. The model was used to identify an appropriate HRT and depth for a PSBR and to elucidate the effect of seasonal light availability on nitrogen removal performance. A full-scale PSBR could achieve >90% ammonia removal, significantly reducing the nitrogen load to the mainstream wastewater treatment plant. The dynamic simulation showed that the ABSNR process can help wastewater treatment facilities meet stringent nitrogen removal standards with low energy inputs.

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DISCLOSURE STATEMENT

The authors report no commercial or proprietary interest in any product or concept discussed in this article.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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