

**A Novel Mechanistic Model for Nitrogen Removal in Algal-Bacterial Photo
Sequencing Batch Reactors**

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Abstract

A comprehensive mathematical model was constructed to evaluate the complex substrate and microbial interaction in algal-bacterial photo sequencing batch reactors (PSBR). The kinetics of metabolite, growth and endogenous respiration of ammonia oxidizing bacteria, nitrite oxidizing bacteria and heterotrophic bacteria were coupled to those of microalgae and then embedded into widely-used activated sludge model series. The impact of light intensity was considered for microalgae growth, while the effect of inorganic carbon was considered for each microorganism. The integrated model framework was assessed using experimental data from algal-bacterial consortia performing sidestream nitrification/denitrification. The validity of the model was further evaluated based on dataset from PSBR performing mainstream nitrification. The developed model could satisfactorily capture the dynamics of microbial populations and substrates under different operational conditions (i.e. feeding, carbon dosing and illuminating mode, light intensity, influent ammonium concentration), which might serve as a powerful tool for optimizing the novel algal-bacterial nitrogen removal processes.

Keywords: Photosynthetic aeration; photo sequencing batch reactor; mathematical model; algal-bacterial consortia; nitrification/denitrification

1. Introduction

To achieve energy autarky or even positive from wastewater treatment, new techniques are applied to reduce the energy consumption. Considering that aeration accounts for 45-75% of energy consumption in wastewater treatment plants (WWTPs)

(Stenstrom and Rosso, 2008), photosynthetic aeration, potentially capable of replacing mechanical aeration, has become a research hotspot. In treatment systems with consortia of microalgae and bacteria such as high-rate algal ponds (HRAP) (Munoz and Guieysse, 2006), microalgae provides the required oxygen (O_2) through photosynthesis process for organic matter (COD) removal by aerobic bacteria. This synergistic interaction not only leads to energy savings, but also limits the release of volatile organic compounds and airborne microbial content into atmosphere (Hamoda, 2006). The produced microalgae biomass could be diverted to the sludge line, to generate biofuels such as biogas, biodiesel and bioethanol (Park et al., 2011).

Nitrogen (N) removal by HRAP is limited by seasonal temperature variation or pH inhibition (Nurdogan and Oswald, 1995; Craggs et al., 2003). In comparison, enclosed photo bioreactors with different configurations, such as flat-plate bioreactors (Zhang et al., 2015), bubble column reactors (Béchet et al., 2013), tubular reactors (Rubio et al., 1999), oscillatory baffled reactor (Abbott et al., 2015), are advantageous in their higher photosynthetic efficiency, better control and robustness. De Godos et al. (2014) reported over 90% of carbon and nitrogen removal and enhanced biomass sedimentation in an innovative anoxic-aerobic photo bioreactor with internal and external recyclings. Liu et al. (2017) successfully cultivated algae-bacteria granular consortia by selection pressure control with maximum N removal rate discovered at highest algae concentration. The application of photo bioreactors with algal-bacterial consortia coupled to biological nitrogen removal has shown great potential in improved removal efficiency and decreased energy consumption (Karya et al., 2013; Boelee et al., 2014; Wang et al., 2015). In these studies, nitrogen removal was obtained by biomass assimilation,

conventional nitrification/denitrification process or shortcut nitrogen removal process.

Mathematical modeling serves as a powerful tool to study the complex interactions among bacteria, algae and growth substrates as well as the effect of varying environmental factors on the performance of the photo bioreactor. Several kinetic models have been developed to characterize algal growth under varying conditions of light intensity (Yuan et al., 2014; Koller et al., 2017), nitrogen levels (Mairet et al., 2011), and temperature (Franz et al., 2012). To facilitate practical application of mathematical model on forecasting microalgae activity, the River Water Quality Model 1 (RWQM1) was proposed and constructed by the International Water Association (IWA) (Reichert et al., 2001), which belongs to the widely-accepted IWA family models (i.e. Activate Sludge Models and Anaerobic Digestion Models). Based on these model frameworks, an integrated model considering multiple affecting factors was developed to describe and predict microalgae growth and production in wastewater (Solimeno et al., 2015). Furthermore, algal-bacterial models were developed to simulate algal-bacterial growth in photobioreactor (Zambrano et al., 2016; Arashiro et al., 2017; Shriwastav et al., 2018). However, complex interaction between microalgae and bacteria and the impact of operational conditions on nitrogen removal in the algal-bacterial systems are yet to be further studied.

The aim of this study is to propose a new model framework to provide insights into the microbial and substrate interactions in the algal-bacterial photo sequencing batch reactors (PSBR). Experimental data from PSBR performing nitrification-denitrification under different operational conditions were used to calibrate and validate the proposed model kinetics. To further assess its validity and predictive ability, the model with

calibrated parameter values was evaluated using experimental data from PSBR performing full nitrification. The proposed model will fill the research gap relevant to modeling mixed cultures of microalgae and bacteria treating wastewater and optimizing operational conditions for high N removal in such systems.

2. Materials and Methods

2.1. Development of a Comprehensive Model for Algal-Bacterial PSBR

The proposed model considers the co-existence of phototrophic, aerobic and anoxic growing microorganisms to achieve an energy-efficient and environmental-friendly nitrogen removal from wastewater in algal-bacterial PSBR. During the daytime, microalgae grow on light and growth substrates concomitant with the release of oxygen, which is simultaneously utilized by aerobic microbes. During the night, the oxygen is depleted rapidly in the absence of photosynthesis and then the anoxic denitrification processes occur. The model framework is developed according to the structure of the existing Activated Sludge Model (ASM) series by International Water Association to facilitate its application. Specifically, model state variables characterizing organic carbon, dissolved O_2 , N and alkalinity (ALK) cycling in the proposed model can be readily linked to the ASM. Multiple-substrate Monod kinetics is uniformly applied for both growth and endogenous respiration processes. The soluble substrates include ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrogen gas (N_2), soluble COD (S_S), ALK and hydrogen ions (H^+). The solid substrates are ammonia oxidizing bacteria (AOB), nitrite

oxidizing bacteria (NOB), heterotrophic bacteria (HB), microalgae biomass (ALG), slowly biodegradable biomass (X_S) and inert biomass (X_I).

The model framework consists of 18 biotic and physical processes describing the growth, metabolite and endogenous respiration of AOB, NOB, HB and ALG. A modified ASM No. 3 (ASM3) with two-step nitrification and denitrification by Iacopozzi et al. (2007) is adapted to describe N removal under nitrification or nitrification conditions. In comparison to ASM No. 1 by Henze, et al. (2000) with single-step nitrification and denitrification, the autotrophic nitrification is modeled by two separate kinetics for the AOB and NOB. NH_4^+ is firstly oxidized to NO_2^- by AOB, with NH_4^+ and dissolved oxygen (DO) as limiting factors. NO_2^- is subsequently oxidized to NO_3^- by NOB. Regarding the process of heterotrophic denitrification, the denitrifiers utilize organic carbon under anoxic condition coupled to either nitrate reduction or nitrite reduction. During nitrification/denitrification processes, NOB are out selected leading to short-cut pathway from NH_4^+ to NO_2^- by AOB and NO_2^- to N_2 by denitrifiers. The kinetic rates for these processes are shown in the E-supplementary data.

Microalgae growth is affected by multiple factors such as the availability of carbon dioxide, light intensity, and temperature. The growth rate of microalgae biomass is modeled as the product of their maximum specific growth rate by their concentration at that point in time and by corrective factors (in the form of Monod functions) that limit their growth. Microalgae utilize inorganic carbon (both carbon dioxide and bicarbonate) as carbon source and $\text{NH}_4^+/\text{NO}_3^-$ as nitrogen source for cell synthesis, while their energy metabolism is dependent on photosynthesis. The microalgae growth rate increases with increasing photon flux density (I) until reaching the optimum (Koller et al., 2017), but

ceases in the absence of light. In terms of kinetics relevant to microalgae growth, the model have considered three key factors, three growth substrates (NH_4^+ , NO_3^- and inorganic carbon) and the light intensity (I), which has been incorporated into the following non-steady-state equation:

$$\frac{dX_{ALG}}{dt} = \mu_{ALG} f(I) \frac{S_{ALK}}{S_{ALK} + K_C^{ALG}} \frac{S_N}{S_N + K_N^{ALG}} X_{ALG} \quad (1)$$

where μ_{ALG} represents the maximum growth rate of microalgae, d^{-1} ; $f(I)$ represents the light intensity function; S_{ALK} represents the concentration of alkalinity, $\text{mmol HCO}_3^-/\text{L}$; K_C^{ALG} is affinity constant of microalgae on carbon species, $\text{mmol HCO}_3^-/\text{L}$; S_N is the concentration of NH_4^+ or NO_3^- , mg N/L ; K_N^{ALG} represents the affinity constant of microalgae on nitrogen species; and X_{ALG} is the biomass concentration of ALG, mg COD/L .

The effect of light intensity on ALG growth was described by $f(I)$ as proposed by Webb (1969), which considers the photoinhibition, i.e., the photosynthetic rate of microalgae is proportional to light intensity at low light irradiance. However, as the intensity continues to increase, the photosynthesis will gradually saturate and its rate even begins to drop after reaching the inhibition threshold. The kinetic rate is detailed in the following equation:

$$f(I) = \frac{I \cdot (1 + \frac{\beta \cdot I}{K_I})}{I + K_S + \frac{I^2}{K_I}} \quad (2)$$

where β is empirical parameter; K_S is light saturation constant, $\mu\text{mol m}^{-2} \text{s}^{-1}$; K_I is light inhibition constant, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

It should be noted that the model has made several assumptions: i) to better integrate the ALG kinetics into ASM-based model framework, the carbon chemical equilibrium is

not specifically considered. Alkalinity is used to represent inorganic carbon species in the developed model; ii) A temperature-constant mode is applied, since the investigated bioreactor is enriched in temperature-controlled room; iii) the CO₂ inhibition is not taken into account since the excess CO₂ will be released into atmosphere; iv) in reality, light is not homogenously distributed within the reactor, however the light absorption or scattering is not modeled for simplicity.

In the model framework, the interaction between microalgae and bacteria was simulated by the synergic and competitive growth kinetics. In terms of synergic growth kinetics, microalgae produce oxygen for aerobic growing bacteria (Supplementary Material). The following equation describes oxygen provide rate by microalgae growth on ammonia.

$$OP = \left(\frac{8i_{C,ALG}}{3} + 8i_{H,ALG} - i_{O,ALG} - \frac{12i_{N,ALG}}{7} \right) \mu_{ALG} f(I) \frac{S_{ALK}}{S_{ALK} + K_C^{ALG}} \frac{S_{NH_4}}{S_{NH_4} + K_N^{ALG}} X_{ALG} \quad (3)$$

Where OP is oxygen provide rate; $i_{C,ALG}$, fraction of carbon in microalgae, gC gCOD⁻¹; $i_{H,ALG}$, fraction of hydrogen in microalgae, gH gCOD⁻¹; $i_{O,ALG}$, fraction of oxygen in microalgae, gO₂ gCOD⁻¹; $i_{N,ALG}$, fraction of nitrogen in microalgae, gN gCOD⁻¹.

The produced oxygen by microalgae is utilized by AOB, NOB and HB for aerobic growth. The following equation shows AOB growth rate as an example.

$$GR_{AOB} = \mu_{AOB} \frac{S_{NH_4}}{S_{NH_4} + K_{NH_4}^{AOB}} \frac{S_{O_2}}{S_{O_2} + K_{O_2}^{AOB}} \frac{S_{ALK}}{S_{ALK} + K_{ALK}^{AOB}} X_{AOB} \quad (4)$$

Where GR_{AOB} is AOB growth rate; μ_{AOB} is maximum growth rate of AOB, d⁻¹; $K_{NH_4}^{AOB}$ is NH₄ affinity constant for AOB, g N m⁻³; $K_{O_2}^{AOB}$ is oxygen affinity constant for AOB, g O₂ m⁻³; K_{ALK}^{AOB} is alkalinity affinity constant for AOB, mol HCO₃⁻ m⁻³.

As for competitive growth kinetics, the microalgae and bacteria compete for the

common growth substrates. For instance, AOB and microalgae compete for ammonia and alkalinity. HB and microalgae compete for nitrate and alkalinity. Their competitive ability is defined by the corresponding growth rate, which is modeled as the product of their maximum specific growth rate by their concentration at that point in time and by corrective factors that limit their growth (growth substrates, see equation above). In sum, the interaction between bacteria and microalgae was modeled as synergic or competitive utilization of the common substrates for their growth.

2.2. Experimental Data in Literature for Testing the Model

Experimental data in bench-scale PSBR from two different studies under nitrification and nitrification conditions, respectively, (Karya et al., 2013; Wang et al., 2015) were used to calibrate and validate the kinetic parameters and the developed model. The growth of microalgae, nitrifiers and heterotrophs coupled to N assimilation and biological N removal was described by the proposed model. The experimental approaches are briefly summarized as below.

Nitrification-PSBR: a lab-scale algal-bacterial PSBR was operated at a climate-controlled room. The cycle time is 24 h/cycle consisting of feed, react, settle and decant. The hydraulic retention time (HRT) and solid retention time (SRT) were maintained at approximately 4 days and 8 days, respectively. The PSBR was continuously illuminated at light irradiance of $74 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ using white fluorescent tubes during start-up period (day 0-25). After reaching steady performance of nitrification, the continuous illumination was replaced by alternate 12h light/12h dark mode. Since day 50, the light intensity was increased to $105 \pm 7 \mu\text{mol m}^{-2} \text{s}^{-1}$. In terms of additional carbon dosage, the

PSBR was operated in the absence of carbon supplement from day 0-80 and in the presence of sodium acetate from Day 90-163. More details on the experimental methodology refer to Wang et al. (2015). The reactor data from day 0-80 and experimental data from one typical cycle during this period were used to calibrate the model and evaluate its predictive ability for the impact of varying illuminating modes and light intensity on reactor performance. The reactor data from day 90-163 and experimental data from two typical cycles were used to validate the model with estimated parameter values and evaluate its robustness of describing reactor performance under heterotrophic conditions with different external carbon dosing modes.

Nitrification-PSBR: an algal-bacterial PSBR with working volume of 1 L was used to perform complete nitrification utilizing the oxygen produced by microalgae under constant temperature of 30 °C and pH around 7.5. Two lamps were placed at the opposite sides of the bioreactor, resulting in a light irradiance of $63 \mu\text{mol m}^{-2} \text{s}^{-1}$. The PSBR was operated under different conditions, such as variation in the number of cycles per day, irradiance, influent alkalinity, SRT etc. Further details can be found in Karya et al. (2013). The long-term operation data and one-cycle data were used to further evaluate the proposed model in description of the substrate conversion in the PSBR under mainstream nitrification conditions.

2.3. Calibration and Validation of the Developed Model

The developed model contains 47 stoichiometric and kinetic parameters, as summarized in E-supplementary data. The parameters relevant to AOB, NOB and HB kinetics were adapted from literature such as the well-established ASMs by international

water association (Henze et al., 2000). A sensitivity analysis was then conducted to identify the most sensitive parameters based on AQUASIM built-in algorithms (absolute-relative sensitivity function as shown below) (Reichert, 1998).

$$\delta_{y,p} = p \frac{\partial y}{\partial p} \quad (5)$$

Where y is an arbitrary variable calculated by AQUASIM and p is a model parameter represented by a constant variable. The absolute-relative sensitivity function measures the absolute change in y for a 100% change in p .

The model calibration involved optimizing these key parameter values by fitting simulation results to long-term operation profiles under varying operational conditions. The AQUASIM 2.1 software was used to perform the estimation of parameters and the calibration of the model (Reichert, 1998), using the completely mixed reactor compartment module to simulate the suspended-growing consortia of microalgae and bacteria. Parameters in the proposed mathematical model were estimated by minimizing the sum of the square of the weighted deviations between measurements and calculated model results for dynamic simulation. The objective function to be minimized in the parameter estimation is as follows:

$$F^2(p) = \sum_{i=1}^n \left(\frac{y_{m,i} - y_i(p)}{\sigma_{m,i}} \right)^2 \quad (6)$$

where $y_{m,i}$ is the measured data at time t_i (i from 1 to n); $y_i(p)$ is the calculated value by the model at time t_i (i from 1 to n); p is the parameters to be estimated; $\sigma_{m,i}$ is the standard deviation of the measurement. With the built-in simplex and secant algorithms, at each iteration, parameter arrays were replaced by new values until $F^2(p)$ are close enough to fulfill the convergence criterion. The details for the numerical integration procedures refer to Reichert, (1998).

Experimental data from Nitritation-PSBR under sidestream conditions and from Nitrification-PSBR under mainstream conditions were both used to evaluate the proposed model framework. The long-term operation data from Nitritation-PSBR and the dynamic profiles within one cycle were used to estimate these key parameters. Then the long-term operation data from Nitrification-PSBR were further used to evaluate the obtained parameter values.

3. Results and discussion

3.1. Model Calibration

The developed model considered the effect of inorganic carbon on the growth and decay of AOB, NOB, HB and ALG, with the parameter alkalinity being used to replace inorganic carbon. On one hand, alkalinity is a very common parameter in WWTP, mostly contributed by inorganic carbon (Peng et al., 2016b). On the other hand, by using alkalinity, the kinetics related to metabolite and growth of microalgae were integrated into ASM No.3 to facilitate future application. Solimeno et al. (2015) has considered inorganic carbon as a limiting factor on microalgae growth since the inorganic carbon in HRAP is usually limited. The developed model implemented the carbon limitation into the model framework through introducing several correction factors K_{ALK}^{AOB} , K_{ALK}^{NOB} , K_{ALK}^{HB} and K_C^{ALG} in the equations describing the growth and decay rate of AOB, NOB, HB and microalgae, respectively. Kurano and Miyachi (2005) has involved the inhibitory effect of excessively high concentrations of inorganic carbon on microalgae growth, which was though not taken this into account in this work due to the fact that excess of CO₂ would

be released to the atmosphere in both bench-scale reactor and full-scale HRAP.

The proposed model framework also considered the impact of light intensity on microalgae growth and system performance. In essence, it is commonly known that light exerts a predominant influence on microalgae growth in condition that nutrients are non-limiting (Koller et al., 2017). From the point view of mathematical modeling, the growth rate of microalgae increases with increasing light intensity and at saturating light intensity photoinhibition cause decreasing growth rate (Huesemann et al., 2013). The kinetics developed by Webb (1969) took into account both photosynthesis and photoinhibition. This approach was adopted in modeling microalgae growth in this work (Equation 1 and 2). The present model considered the main aspects of the photosynthetic response of algal-bacterial consortia to light. However, the growth rate of microalgae may vary from point to point in PSBR since the light is not homogenously distributed within the reactor in reality (Cornet et al., 1992). In addition to light scattering, the pigment absorption and the shading effect of microalgae would also influence light attenuation and thus affect the microalgae growth (Solimeno et al., 2015). Considering the scarce data available and requirement to reduce complexity of model simulation, the developed model did not involve these aspects specifically. Nevertheless, the current model framework is adequate to predict system performance and microbial interaction for algal-bacterial consortia performing nitrification/denitrification under sidestream conditions as well as nitrification under mainstream conditions.

A sensitivity analysis was performed to test the impact of key parameters on the model output in terms of N conversion. Based on AQUASIM built-in algorithms (absolute-relative sensitivity function), the top sensitive parameters have been identified

as maximum growth rate of AOB (μ_{AOB}), maximum growth rate of microalgae (μ_{ALG}), affinity constant of microalgae on carbon species ($K_{C,ALG}$) and parameters in the light intensity function (β , K_I , K_S). Hence, these parameters were calibrated while the remaining well-established parameters were the same as literature (details in E-supplementary data). The obtained value of μ_{AOB} is 0.82 d^{-1} , which is higher than the reported value in ASM series (0.45 d^{-1} , Henze et al., 2000), but lower than that in Wiesmann (1994) (2.05 d^{-1}). These parameter values are in comparable range and the variation is likely because of the difference regarding the aeration mode and the involved microorganisms between the novel algal-bacterial system and the conventional system. The calibrated values of μ_{ALG} (0.91 d^{-1} for nitritation and 0.51 d^{-1} for nitrification) are in good agreement with the literature ranges of 0.4 to 2 d^{-1} (Reichert et al., 2001). The obtained value of $K_{C,ALG}$ is much higher than that in Novak and Brune (1985). This is because that ALG are the sole microorganism utilizing inorganic carbon in the study by Novak and Brune (1985), whereas AOB, NOB, HB and ALG consume/produce the common substrate in the investigated system. The corresponding affinity constants determine consumption/production rate of inorganic carbon in the complex co-culture system. Through calibration, it has been revealed that the estimated value of $K_{C,ALG}$ is similar to the values of K_{ALK}^{AOB} , K_{ALK}^{NOB} and K_{ALK}^{HB} adapted from literature (Iacopozzi et al., 2007) (E-supplementary data). The estimated relevant parameter values for β , K_I and K_S are 0.07 , $747.1 \mu\text{mmol m}^{-2} \text{ s}^{-1}$ and $48.3 \mu\text{mmol m}^{-2} \text{ s}^{-1}$, respectively, which are comparable to the parameter values (0.19 , $1538 \mu\text{mmol m}^{-2} \text{ s}^{-1}$ and $39 \mu\text{mmol m}^{-2} \text{ s}^{-1}$) using the same light intensity function for the description of light impact on photo bioreactor (Koller et al., 2017).

3.2. Model Evaluation in Nitrification-PSBR under Sidestream Conditions

The calibration of the developed model involved optimizing key parameter values for conversions of N, COD, DO and ALK as well as their interaction with biomass growth by fitting simulation results to experimental data under different conditions. The calibrated parameter values giving the optimum fit are listed in E-supplementary data. The experimental observation and model simulation results of biomass, influent and effluent NH_4^+ , effluent NO_2^- during long-term operation of the Nitrification-PSBR without external carbon supplement are shown in the Figure 1. The light intensity was $74 \mu\text{mol m}^{-2} \text{s}^{-1}$ from day 0 to day 50 and was shifted to $105 \mu\text{mol m}^{-2} \text{s}^{-1}$ since day 51. The model with estimated parameters managed to adapt to the increasing total biomass concentration upon increasing light intensity (Figure 1A). Furthermore, the model was able to predict the shift of different microbial community. The increased light intensity induced the increased biomass concentrations of ALG and AOB, while the biomass concentration of HB almost remained the same (Figure 1A). The model further predicted that ALG were the most dominant biomass over AOB and HB. The model simulation results also fitted well with measured influent and effluent variations (Figure 1B&C). The increased light intensity resulted in lower effluent NH_4^+ concentration, but higher effluent NO_2^- concentration via higher DO supplement. Through model simulation, it has been found that the photoinhibition occurs when light intensity is over $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the light intensity in this study is far below this threshold.

Model calibration also involved matching profiles of NH_4^+ , NO_2^- , DO, BOD_5 and ALK in PSBR within one cycle of 24 hours (Figure 2). The experimental data were

measured after the reactor reaching steady state under alternate 12h light/ 12h dark illuminating mode and in the presence of additional carbon. During darkness period (0-10h and 22-24h), the initial increase of NH_4^+ concentration and decrease of NO_2^- concentration in the reactor were due to feeding. After feeding, NH_4^+ oxidation barely took place due to lack of oxygen. NO_2^- concentration slightly decreased due to heterotrophic denitrification. When light was provided, ALG started to produce oxygen for ammonia oxidation leading to the decreased NH_4^+ and increased NO_2^- . The proposed model managed to match the dynamics of NH_4^+ and NO_2^- (Figure 2A). There was a discrepancy between measured DO and model prediction during dark period (0-10 hour and 22-24 hour) (Figure 2B). The DO concentration was around 0.2 mg O_2/L , while the simulated DO concentration was close to zero. In the whole cycle, BOD_5 showed a decreasing trend (Figure 2C), while alkalinity followed the similar changing pattern with nitrogen (Figure 2D). The dynamics of BOD_5 and alkalinity could be reproduced by the model.

The observed and predicted profiles of ammonia and nitrite concentration as well as alkalinity in Figure 2A&D were almost stable during dark period, which indicated that AOB were inactivated due to a lack of available oxygen during dark period. The model with calibrated parameters here gives the optimal fit against experimental data and the discrepancy between simulation and measurement in terms of DO profiles during dark period is possibly caused by the measurement accuracy. The relatively constant DO concentration in bulk solution results from a balanced oxygen supply and consumption by the algal-bacterial consortium in the biomass flocs. Despite of the discrepancy of DO profile during dark period, the proposed model could describe the DO transition during

the shift from dark to light and reproduced the balanced DO level during light period.

It is noted that the model could only simulate DO concentration during light period (Figure 2B). The discrepancy between measured DO and model prediction during dark period is probably because of the introduction of oxygen by influent feeding during the experiments. Due to the limited information, the model did not consider this factor.

To test the validity and reliability of the proposed model with the calibrated parameters, its simulation results were compared to another set of long-term experimental data under different conditions (i.e., the presence of the external carbon source, different feeding and carbon dosing modes, etc.). Figure 3 shows the measured and simulated data of biomass, influent and effluent with feeding of centrate and external carbon concurrently in darkness (day 90 to day 130) and with separate supplement of centrate and external carbon in light period and dark period, respectively (day 130 to day 165). The change of feeding and external carbon dosing from combined mode to separate mode largely increased the total biomass concentrations and improved nitrite reduction, while AOB and NH_4^+ concentrations almost kept unchanged. The proposed model managed to describe the dynamics of biomass and N upon the change of feeding mode and further predicted the biomass concentration of AOB, HB and ALG. As observed in experiments (Figure 1A and 3A), light intensity influenced total biomass concentration in the investigated system. Further model simulation with calibrated parameters revealed that increase of light intensity directly elevated the biomass level of microalgae and favored the growth of AOB and HB via increased oxygen supplement (Figure 1A and 3A). This further confirmed the validity of the developed model framework.

The model validation results on nitrogen profiles within one operational cycle (24

hours) are shown in Figure 4. When feeding centrate and sodium acetate concurrently at the beginning of dark period (Figure 4A), NH_4^+ was stable in most of the dark period (0-12h) except the initial feeding phase. NO_2^- was decreasing due to feeding dilution at the beginning and denitrification in the dark period. In the following light period, NH_4^+ was converted to NO_2^- utilizing the oxygen produced by ALG. When feeding centrate at the beginning of light period (0 - 12h) and dosing sodium acetate in the darkness from hour 12 to hour 24 (Figure 4B), NH_4^+ was firstly converted to NO_2^- in light period. Subsequently, the produced NO_2^- was completely denitrified to N_2 gas. The nitrogen concentration in the effluent is close to zero. The simulation results demonstrate that the model has a good predictive ability to describe nitrogen dynamics in the two feeding modes.

3.3. Model Evaluation in Nitrification-PSBR under Mainstream Conditions

To demonstrate the broad applicability of the proposed model, it was further tested against nitrification data under mainstream conditions. The most sensitive parameter μ_{ALG} was re-calibrated. The details are shown in E-supplementary data. Figure 5 and Figure 6 presents the model evaluation results in long-term operation and in one representative cycle, respectively. Despite of several NH_4^+ peaks in the middle of the operation, effluent NH_4^+ concentration was close to zero, indicating a very high NH_4^+ removal efficiency (Figure 5A). The NOB was very active since the NO_2^- accumulation was only observed on day 40. Almost all of the NH_4^+ was oxidized to NO_3^- (Figure 5B). Within one cycle of 24 hours, light was continuously provided at light intensity of $63\mu\text{mol m}^{-2} \text{ s}^{-1}$. The model prediction matched the dynamics of NH_4^+ , NO_2^- , NO_3^- and

DO. The agreement indicated that the proposed model could be applied in nitrification PSBR under mainstream conditions. Figure 6 showed that the simulation results by the proposed model were also in good agreement with measured data in terms of NH_4^+ , NO_2^- , NO_3^- and DO concentrations within one typical cycle, demonstrating the good predictive ability and validity of the model for mainstream wastewater treatment. There are some discrepancies at hour 6 as can be seen in Figure 6B. This is possibly due to the overshooting signal of DO sensor especially during the start of photosynthetic aeration. However, the overall trend is satisfactory. Both predicted and measured DO was completely depleted in the first half hour. After light exposure, the DO concentration rapidly increased and the final DO set points from prediction and measurement both reached around 8 mg O_2/L at 10th hour.

3.4. Implications

In this work, a comprehensive model framework for describing microbial interaction and N mechanism in novel algal-bacterial system was proposed. Photo sequencing batch reactor was used to co-culture AOB, NOB, HB and ALG, where their metabolite, growth and endogenous respiration were individually modeled and incorporated into the ASM framework by international water association. The photosynthetic aeration in the system results in large energy savings (Stenstrom and Rosso, 2008), and its combination with shortcut N removal process further decreases the operational cost of WWTPs. The proposed model was well calibrated and validated with experimental dataset under highly different conditions. It has been demonstrated that the model managed to predict nitrogen removal and microbial community shift in both

nitritation/denitritation and nitrification systems. Thus, the developed mathematical model might be used as a predictive tool to provide useful information for design and operation of PSBR under both sidestream and mainstream conditions.

Several algal-bacterial models that have been proposed previously, contain plenty of limitations. The model by Zambrano et al. (2016) coupled one-step nitrification to algal growth, however the reliability and robustness of the model are yet to be tested using long-term dataset and under mixotrophic conditions. The model by Shriwastav et al. (2018) didn't consider the light impact on microalgae growth. The NO_3^- -rich influent (less relevant to real wastewater), the lack of nitrification/denitrification process and the complex model structure (37 processes) brought difficult to application. The model by Arashiro et al. (2017) made oversimplified assumptions, which neglected the impact of inorganic carbon on microbial growth and NO_3^- assimilation by microalgae. The endogenous respiration on NH_4^+ during the dark periods was not properly modeled. To solve the above-mentioned issues, the model took into account all common practical variables (different nitrogen, carbon species and light intensity) and microbial processes (growth and endogenous respiration of AOB, NOB, HB and ALG) with the aim to mimic scenario of real wastewater. The parameters in the model were well evaluated by different conditions (mode of feeding, carbon dosing and illuminating, autotrophic vs mixotrophic, sidestream vs mainstream and batch vs long-term). Hence, The proposed model in this work possesses a very good predictive ability for real scenarios and moves one step forward to understand the complex processes of algal-bacteria consortia and facilitate future application.

Several following steps might be considered to further complete and improve the

model for future application with the aim for an overall sustainable wastewater treatment. In particular, the developed model framework could be easily modified to incorporate kinetics of emerging processes such as anaerobic ammonium oxidation (anammox) and nitrous oxide (N₂O) production (Ni et al., 2014; Peng et al., 2015). As shown in Figure 2B, the oxygen level in nitrification/denitrification system was below 0.4 mg O₂/L, which is favorable for anammox bacteria. In essence, the novel algal-nitrifying bacterial consortium and anammox granules were demonstrated to be feasible in the study by Manser et al. (2016). With the developed model, existing anammox kinetics and available dataset, preliminary simulations could be performed to evaluate the feasibility of novel microalgae+AOB+anammox process under both sidestream and mainstream conditions. The simulation results would help to optimize operational conditions for demonstration in bigger scales. Moreover, N₂O emissions from such novel technology could be predicted through incorporating kinetics relevant to N₂O production pathways (Ni et al., 2014; Peng et al., 2016a; Peng et al., 2017). In terms of full-scale simulations, the current model framework needs to involve flow and transport equations to mimic more complex hydrodynamic and geometric regimes.

4. Conclusion

A comprehensive model was proposed to evaluate nitrogen removal in algal-bacterial photo sequencing batch reactor. The model consists of the metabolite, growth and endogenous respiration of ammonia oxidizing bacteria, nitrite oxidizing bacteria, heterotrophic bacteria and microalgae. The impact of light intensity and carbon limitation on microbial growth was taken into account and assessed with experimental data. The

proposed model could predict nitrogen conversion and microbial interaction in the photo sequencing batch reactors performing nitrification/denitrification under sidestream conditions as well as nitrification under mainstream conditions, with the validity and robustness of the developed model being well demonstrated in this work.

E-supplementary data of this work can be found in online version of the paper.

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Figure Legend

Figure 1. Model calibration: experimentally observed and model predicted results of A) biomass; B) influent and effluent NH_4^+ ; C) effluent NO_2^- during long-term operation of the Nitritation-PSBR without external carbon supplement under different light intensities of $74 \mu\text{mol m}^{-2} \text{s}^{-1}$ (day 0 - day 50) and $105 \mu\text{mol m}^{-2} \text{s}^{-1}$ (day 51 - day 80).

Figure 2. Model calibration: experimentally observed and model predicted results of A) nitrogen; B) DO; C) BOD_5 and D) alkalinity during one cycle of the Nitritation-PSBR without external carbon dosage. Light period is from hour 10 to hour 22 with light intensity of $105 \mu\text{mol m}^{-2} \text{s}^{-1}$, while dark period is remaining.

Figure 3. Model validation results of measured and simulated A) biomass; B) influent and effluent NH_4^+ and C) effluent NO_2^- in Nitritation-PSBR with feeding of centrate and external carbon concurrently in darkness (day 90 to day 130) and with separate supplement of centrate and external carbon in light period (light intensity of $105 \mu\text{mol m}^{-2} \text{s}^{-1}$) and dark period, respectively (day 130 to day 165).

Figure 4. Model validation results of effluent NH_4^+ and NO_2^- from the Nitritation-PSBR with different feeding modes. A) feeding centrate and sodium acetate concurrently at the beginning of dark period from hour 0 to hour 12, while light was provided from hour 12 to hour 22 at light intensity of $105 \mu\text{mol m}^{-2} \text{s}^{-1}$; B) feeding centrate at the beginning of light period from hour 0 to hour 12, while sodium acetate was supplied in the darkness from hour 12 to hour 24.

Figure 5. Model evaluation: A) influent and effluent NH_4^+ ; B) effluent NO_2^- and NO_3^- during long-term operation of Nitrification-PSBR at light intensity of $63 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Figure 6. Model evaluation: A) NH_4^+ & NO_2^- and B) NO_3^- & DO during one cycle operation of Nitrification-PSBR at light intensity of $63 \mu\text{mol m}^{-2} \text{s}^{-1}$.

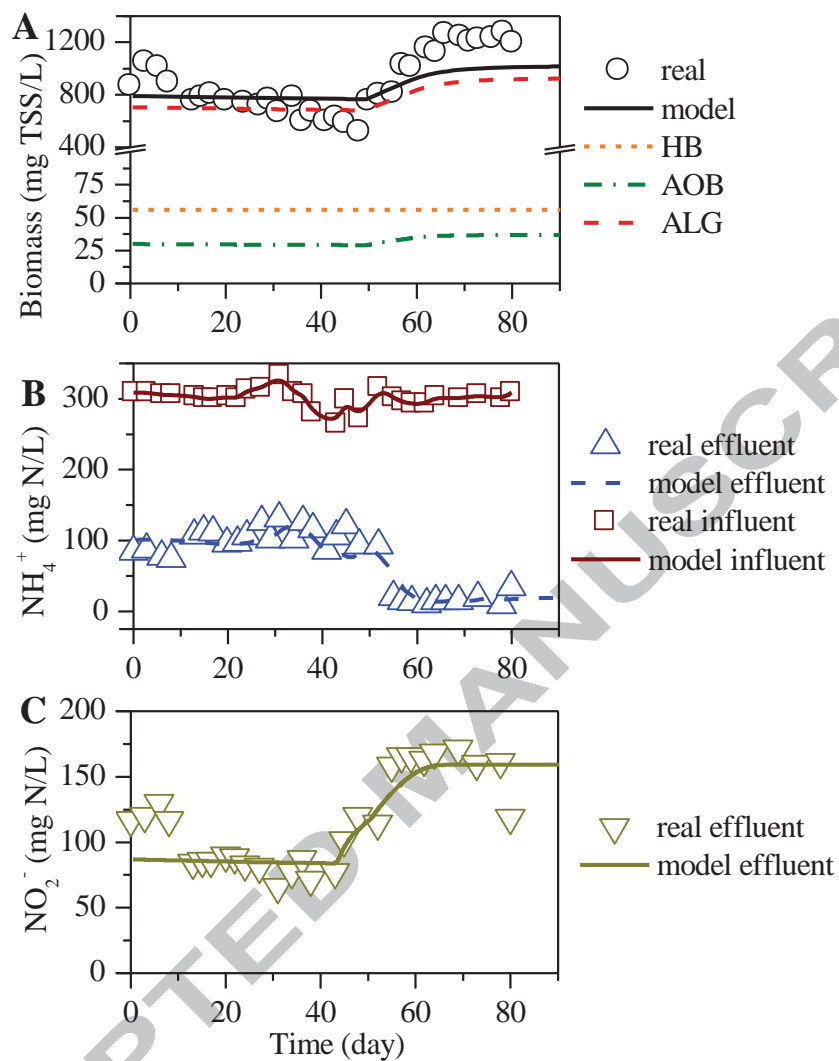


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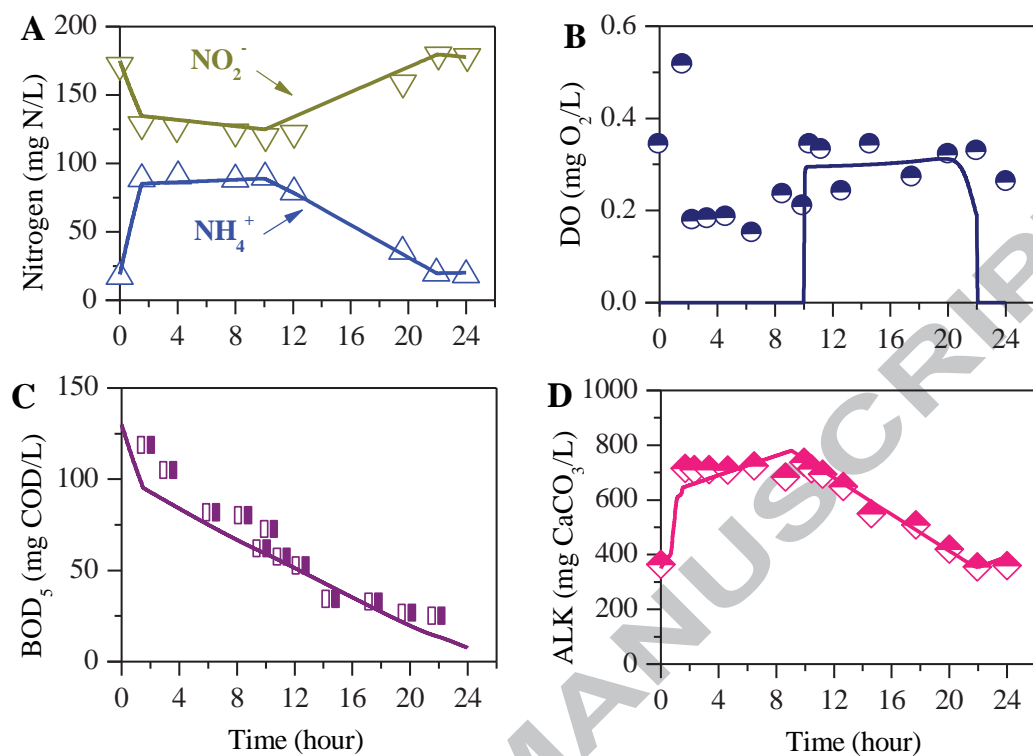


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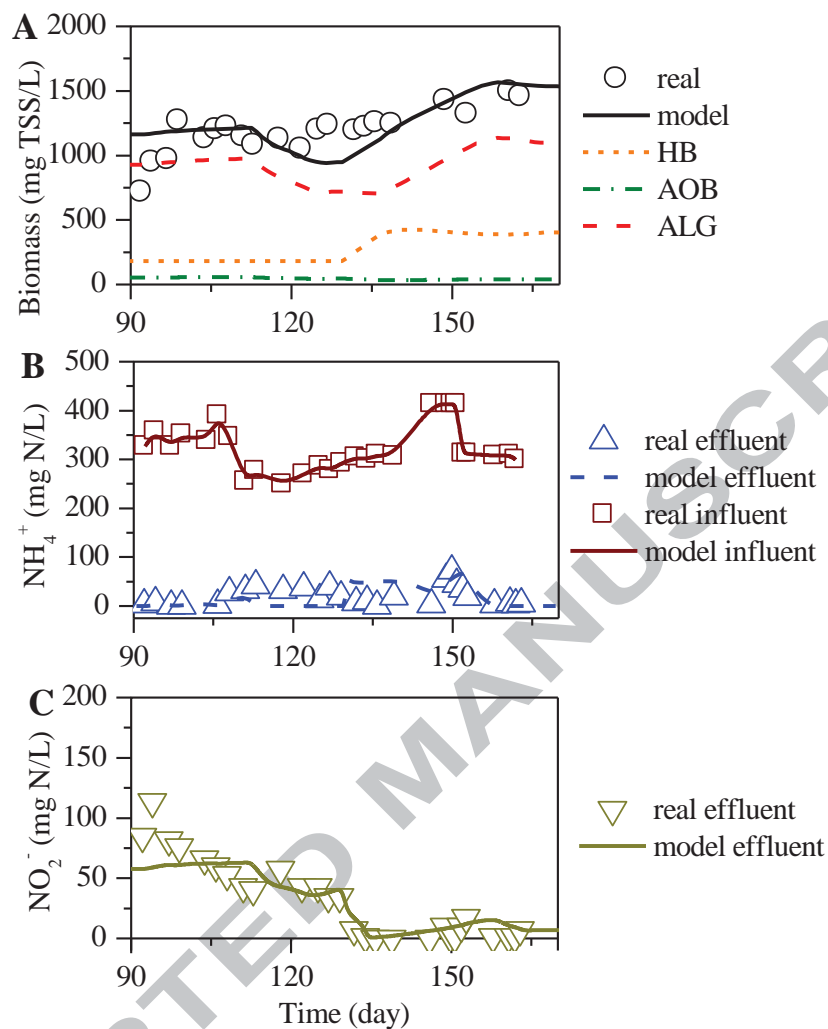


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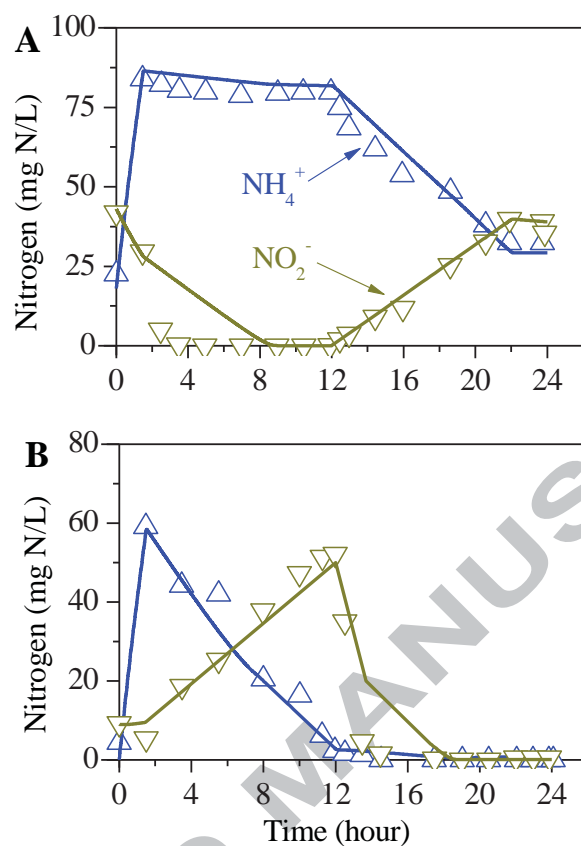


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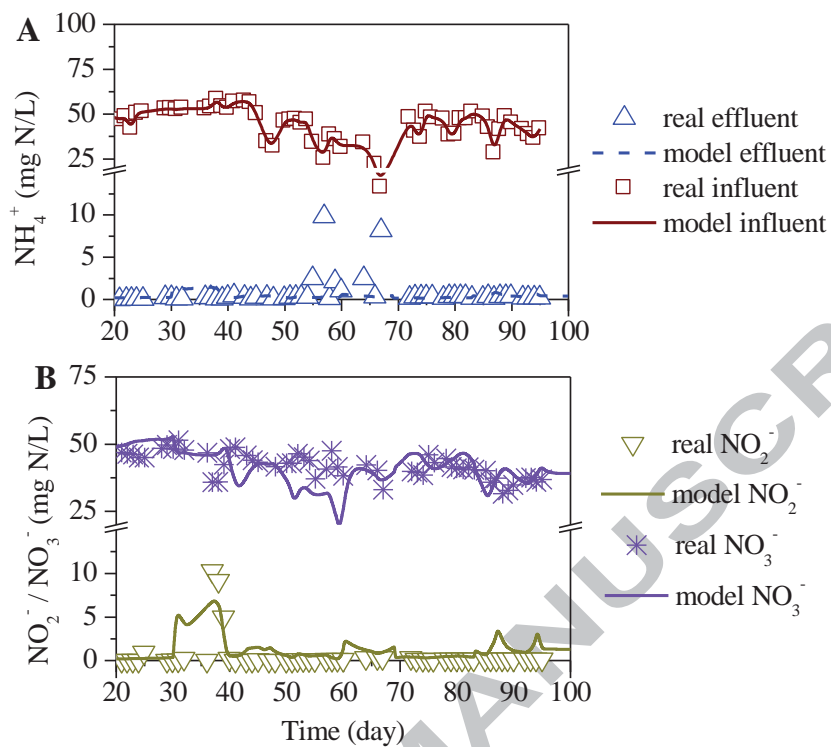


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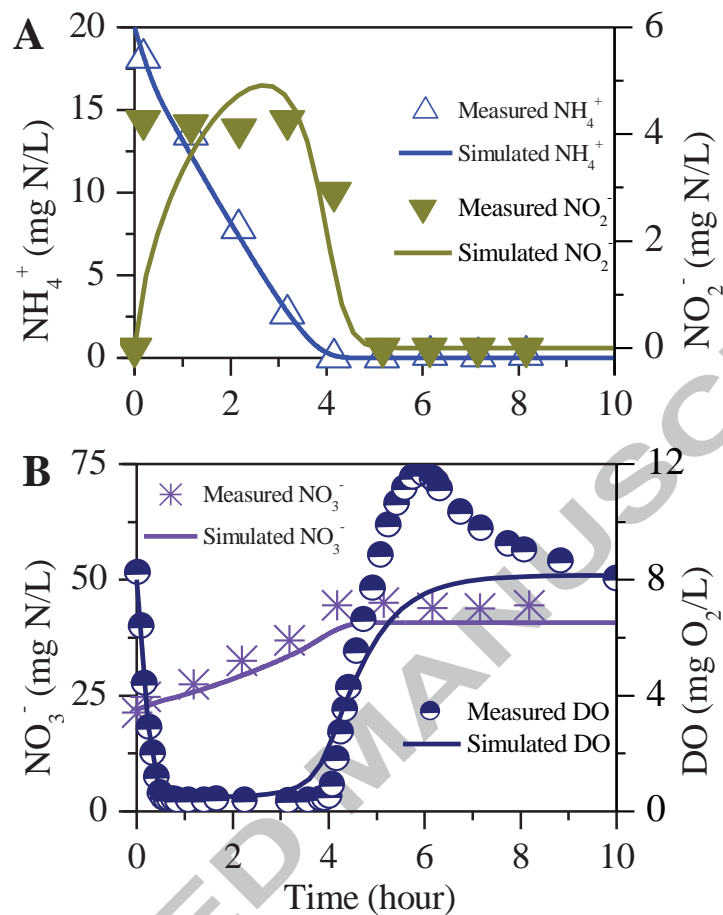


Figure 6. Model evaluation: A) NH_4^+ & NO_2^- and B) NO_3^- & DO during one cycle operation of Nitrification-PSBR at light intensity of $63 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Highlight

- A model was constructed for algal-bacterial photo sequencing batch reactors.
- The kinetics of AOB, NOB and HB were coupled to those of microalgae.
- The model considered variables such as light intensity, inorganic carbon, etc.
- The model was evaluated by experimental data under highly different conditions.

ACCEPTED MANUSCRIPT