Received: 20 January 2025 | Revised: 3 March 2025 | Accepted: 9 March 2025

DOI: 10.1002/wer.70059

RESEARCH ARTICLE

water ENVIRONMENT RESEARCH

Maximizing the efficiency of single-stage partial nitrification/Anammox granule processes and balancing microbial competition using insights of a numerical model study

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Funding information
Ontario Ministry of Research and Innovation, Grant/Award Number: RE09-077; Natural Sciences and Engineering Research Council of Canada, Grant/Award Numbers: RGPIN-2019-06747, RGPAS-2019-00102; National Research Foundation of Korea, Grant/Award Number: 2018H1D3A2065879

Abstract

Granulation is an efficient approach for the rapid growth of anaerobic ammonia oxidation (Anammox) bacteria (X_{ANA}) to limit the growth of nitrite-oxidizing bacteria (X_{NOB}). However, the high sensitivity of Anammox bacteria to operational conditions and the competition with other microorganisms lead to a critical challenge in maintaining sufficient X_{ANA} population. In this study, a one-dimensional steady-state model was developed and calibrated to investigate the kinetic constants of X_{ANA} growth and mass transport in individual granules, including the liquid film. According to the model calibration results, the range of the maximum specific growth rate constant of X_{ANA} (μ_{ANA}) was 0.033 to 0.10 d^{-1}. In addition the other kinetic constants of X_{ANA} were 0.003 d^{-1} for decay rate constant (b_{ANA}), 0.10 mg-O_2/L for oxygen half-saturation constant ($K_{O_2}^{ANA}$), 0.07 mg-N/L for ammonia half-saturation constant ($K_{NH_4}^{ANA}$), and 0.05 mg-N/L for nitrite half-saturation constant ($K_{NO_2}^{ANA}$). The model simulation results showed that the dissolved oxygen of about 0.10 mg-O_2/L was found to be optimal to maintain high X_{ANA} population. In addition, minimal COD concentration is required to control heterotrophs (X_H) and improve ammonia oxidation by ammonia-oxidizing bacteria (X_{AOB}). It was also emphasized that moderate mixing conditions ($L_f \cong 100\,\mu m$) are preferable to decrease the diffusion of oxygen to the deep layers of the granules, controlling the competition between X_{ANA} and X_{NOB}. A single-factor relative sensitivity analysis (RSA) on microbial kinetics revealed that μ_{ANA} is the governing factor in the efficient operation of the single-stage PN/A processes. In addition, it was found that nitrite concentration is a rate-limiting parameter on the success of the process due to the competition between X_{ANA} and X_{NOB}. These findings can be used to enhance our understanding on the importance of microbial competition and mass transport in the single-stage PN/A process.

Practitioner Points:

- A one-dimensional steady-state model was developed and calibrated for simulating the single-stage partial nitrification/Anammox (PN/A) granule process.
- Moderate liquid films ($L_f \cong 100\,\mu m$) are preferable for better performance of Anammox growth in single-stage PN/A processes.
- Moderate dissolved oxygen (DO ≅0.10 mg-O_2/L) is highly recommended for efficient growth of Anammox bacteria in single-stage PN/A granulation.
- Minimal COD (COD ≅0) is preferable for successful operation of the single-stage PN/A granule process.
- Nitrite concentration is a rate-limiting parameter on the competition between Anammox and nitrite-oxidizing bacteria in the single-stage PN/A processes.

KEYWORDS

anaerobic ammonia oxidation (Anammox) bacteria, granulation, microbial communities, mixing conditions, NOB suppression

INTRODUCTION

Elevated concentrations of nitrogen components (e.g., organic nitrogen, ammonia, and nitrate) can be found in multiple wastewater streams such as domestic, agricultural, industrial wastewater, and animal manure waste. Excessive release of nitrogen components into natural water systems (mainly surface water) can cause algal bloom formation and eutrophication in lakes and rivers, threatening the aquatic ecosystem (Ahmed & Lin, 2021; Chen et al., 2020; Elsayed, Rixon, Levison, et al., 2023; Elsayed et al., 2024). Nitrogen components also have a negative influence on human health since they can result in multiple diseases such as methemoglobinemia (i.e., limited oxygen concentration in blood) and cancer (Elsayed, Rixon, Zeuner, et al., 2023; Perović et al., 2020; Rixon et al., 2024; Schullehner et al., 2018). Therefore, the removal of nitrogen components in modern wastewater treatment is extremely important to avoid critical environmental and public health problems.

Anaerobic ammonia oxidation (Anammox) is an innovative technology for nitrogen removal by converting ammonia and nitrite into nitrogen gas without expensive aeration systems and organic demand (Madeira & de Araújo, 2021; You et al., 2020; Yue et al., 2018). This reaction is known as a single-stage partial nitrification/Anammox (PN/A) processes where 45% of ammonia is converted into nitrite while the rest of ammonia (55%) is consumed with the produced nitrite by Anammox bacteria (X_{ANA}) under anaerobic conditions in a single reactor (Antwi et al., 2019; Miao et al., 2016). In addition, the single-stage PN/A process is considered a promising alternative for conventional nitrification and denitrification in municipal wastewater treatment (Cui et al., 2020; Eskicioglu et al., 2018; Weralupitiya et al., 2021) as it requires less aeration cost, less footprint for wastewater treatment facilities and no organic carbon for complete nitrogen removal (Bonassa et al., 2022; Dai et al., 2021; Gao et al., 2023). Also, the single-stage PN/A process has a less sludge production rate coupled with minimal disposal cost and less greenhouse gas emissions (e.g., nitric oxide) compared to the conventional activated sludge process (Conthe et al., 2019; Fu et al., 2023; Wu et al., 2022). Generally, successful operation of the single-stage PN/A processes can save approximately 90% of the operational and running costs compared to the conventional biological nitrogen removal process (Kim & Cui, 2023; Ren et al., 2022; Yang et al., 2021).

However, the growth of X_{ANA} population is relatively slow where the doubling time can be as long as 10 to 20 days depending on the temperature and substrate conditions (Gong et al., 2022; Hu et al., 2022; Waki et al., 2021), resulting in long operational time for successful growth of X_{ANA} population. Also, the partnership between X_{ANA} and ammonia oxidizing bacteria (X_{AOB}) and the competition between X_{ANA} and nitrite oxidizing bacteria (X_{NOB}) for nitrite is a key challenge on efficient growth of X_{ANA} in the single-stage PN/A process. It should be noted that single-stage PN/A processes are more practical compared to the two-stage PN/A processes because of the reduced reactor volume and low nitrous oxide emission with no pH regulations associated with the operation process (Guo et al., 2020a; Guo et al., 2020b). Therefore, the main scope of this study is oriented toward the determination of dominant parameters for the successful operation of the single-stage PN/A processes.

Many previous Anammox process studies investigated the growth of X_{ANA} using granules (Li et al., 2021; Lin et al., 2022; Wang et al., 2020), biofilms (Gilmore et al., 2013; Lotti et al., 2014; Zhang et al., 2014), attached growth systems (Hu et al., 2010; Wang et al., 2009; Zhang et al., 2017) and batch systems (Connan et al., 2016). Granulation is considered one of the most innovative solutions to limit the activity of X_{AOB} and X_{NOB} coupled with providing a sufficient retention time for the growth of X_{ANA} (Ishimoto et al., 2021; Laureni et al., 2015; Lin & Wang, 2017; Xu et al., 2019). Granulation processes also allow shorter start-up and easier control compared to other Anammox growth techniques (Adams et al., 2020; Gonzalez-Gil et al., 2015; Song et al., 2017). In individual Anammox granules within the single-stage PN/A processes, X_{AOB} grow at the outer layers of the granule where dissolved oxygen is provided from the bulk solution. While X_{ANA} grow at the inner (deep) layers of granule where the oxygen mass transfer is limited near the core of the granule.

Operational conditions play a dominant role in the growth of X_{ANA} population using the granulation approaches. In previous experimental Anammox studies, it was observed that dissolved oxygen concentration is an important parameter on the growth of X_{ANA} population as low oxygen concentration is required for better growth of X_{ANA} (Feng et al., 2022; Pérez et al., 2014; Zekker et al., 2019). It was also found in previous Anammox studies that the granule diameter can control the X_{ANA} growth rate (Chen et al., 2019; Dan et al., 2023; Li et al., 2021; Liu, Ma, et al., 2017; Luo et al., 2017; Yuan et al., 2022). C/N ratio (carbon-to-nitrogen) and influent COD concentration are also two dominant parameters on the X_{ANA} growth as they affect the competition between X_{ANA} and heterotrophic bacteria (Gao et al., 2021, 2023; Li et al., 2017; Zhang et al., 2020). The competition between X_{ANA} and other involved microorganisms (i.e., X_{AOB}, X_{NOB} and heterotrophs) can also control the growth of X_{ANA} population (Cao et al., 2017; Pellicer-Nàcher et al., 2013; Wang et al., 2017). However, there are no systematic investigations on the effect of mixing conditions (i.e., liquid film thickness) on the growth of X_{ANA}. In addition, although various studies examined X_{ANA} growth using bench-scale experimentation (Deng et al., 2022; Gong et al., 2022; Su et al., 2023) and mainstream wastewater systems (Liu, Peng, et al., 2021; Liu, Wang, et al., 2021; Zhuang et al., 2022), limited studies investigated the growth of X_{ANA} population in single-stage PN/A granule technology using mathematical models under various operational conditions.

Mathematical models are an important tool for describing the competition between X_{AOB} and X_{ANA} for ammonia, X_{AOB} and X_{NOB} for dissolved oxygen, and X_{ANA} and X_{NOB} for nitrite under various operational conditions (Vannecke et al., 2015; Volcke et al., 2010). Also, these models can be implemented for determining the governing microbial kinetic constants and operational conditions on the diversity of the microbial communities within the single-stage PN/A processes. Mathematical modeling, including various microorganisms (i.e., X_{AOB}, X_{NOB}, heterotrophs, and X_{ANA}), is crucial to detect the complicated correlations between the process parameters (e.g., microbes and operational conditions) (Baeten et al., 2019; Corbalá-Robles et al., 2016). Mathematical models can also be addressed to describe the failure of Anammox operation and challenges in single-stage PN/A processes due to the domination of X_{NOB} with a comprehensive analysis of the failure mechanisms, including rapid growth rate of X_{NOB} compared to X_{ANA} and substrate mass transport to the granule (e.g., granule diameter and liquid film thickness). However, there are no reported mathematical modeling studies to determine the role of microbial kinetic constants of the involved microorganisms on the success of the Anammox operation processes.

In this study, the main objectives are to: (1) develop a one-dimensional mathematical model to simulate the growth of Anammox bacteria (X_{ANA}) in the single-stage PN/A granule processes, (2) calibrate the mathematical model using previous Anammox studies to determine the important model parameters (e.g., kinetics of X_{ANA} and diffusion coefficient of soluble substrates), (3) evaluate the effect of operational conditions (e.g., dissolved oxygen concentration and mixing conditions) on the growth of X_{ANA} population and microbial communities in the single-stage PN/A processes and (4) do a sensitivity analysis on the microbial kinetic constants to assess their effect on the competition between the microorganisms and the X_{ANA} population in the single-stage PN/A processes.

NUMERICAL MODEL DEVELOPMENT AND CALIBRATION

Biological reaction kinetics and mass transport

A one-dimensional steady-state model was developed to simulate the mass transport and the biological reactions driven by X_{ANA}, X_{AOB}, X_{NOB}, and heterotrophic bacteria in a spherical Anammox granule. The model was used to simulate the growth of X_{ANA} based on IWA-ASM3 (International Water Association Activated Sludge Model No.3) (Henze et al., 2000) and the biological reactions of X_{ANA} from previous model studies (Lackner et al., 2008; Ni et al., 2013). For the biological reactions of nitrogenous compounds in the granule, ammonia (S_{NH_4}) was oxidized by X_{ANA} and X_{AOB} into nitrite (S_{NO_2}) that was utilized by X_{ANA} and X_{NOB}. While denitrification reaction was performed by heterotrophic bacteria (X_H) where nitrate (S_{NO_3}) was converted into nitrogen gas (Table S1). Soluble COD (S_{COD}) was utilized by X_H and particulate COD (X_S) was hydrolyzed into S_{COD} for the carbonaceous compounds (Table S1). Microbial decay reactions were also assumed for the particulate components (X_{ANA}, X_{AOB}, X_{NOB}, and X_H) (Table S1).

In the steady state model, the biological reaction kinetics and diffusive mass transport in the granules were included (Table 1), assuming non-homogenous granules in the reactor. In a single granule, the soluble components (S_{NH_4}, S_{NO_2}, S_{NO_3}, S_{O_2}, and S_{COD}) were mobile by diffusion from the bulk solution to the granule through the liquid film (i.e., boundary layer) where biological reactions were assumed to be negligible; however, the particulate components were assumed to be immobile. At the center of granule (r = 0), it was assumed that there was no flux for each of the soluble components (Equation 1).

$$\left.\frac{dS_i}{dr}\right|_{r=0} = 0 \tag{1}$$

where S_i is an individual soluble component and r is the distance from the granule center.

Numerical solution methods

In the steady state model, the 10 mass balance equations for individual soluble and particulate components were discretized using the finite difference method (Table S1) (Chapra & Canale, 1998). In the finite difference method, the granule was evenly divided into 20 grids regardless of the granule size (diameter). The 200 discretized mass-balance equations (20 grids × 10 components) were solved simultaneously by the fixed-point iteration approach (Chapra & Canale, 1998) with a relative tolerance of 0.0001. It should be noted that spherical coordinates (using the radial distance from the granule center [r]) were used in the developed steady-state granule model.

Model calibration

The steady-state granule model was calibrated using simulation results from previous Anammox process modeling studies (Study A (Corbalá-Robles et al., 2016); Study B (Liu, Niu, et al., 2017); Study C (Hao et al., 2002)) to estimate the kinetic constants of Anammox bacteria, the diffusion coefficient of soluble components in the granule and liquid film thickness (Table 2). In the model calibration, the simulation conditions (e.g., soluble components concentration) were assumed based on the mentioned simulation conditions in each of the three studies (Table 2). The simulation results in the literature studies were digitized and extracted to prepare the concentration profiles of soluble and particulate components within the granules.

Relative sensitivity analysis

A single-factor relative sensitivity analysis (RSA) was performed on the kinetic constants of X_{AOB}, X_{NOB}, and X_{ANA} to evaluate their effect on the X_{ANA} population and determine the important kinetics on the growth of X_{ANA}. In the sensitivity analysis, a 1% change in a given kinetic constant was applied and the effect of this change on the Anammox population was evaluated for each discretized grid (20 grids). The average relative sensitivity function (f_{RS}) for a given kinetic constant was described by the rate of change in the Anammox population ($\partial X_{ANA}(r)$) with space (r) normalized by the rate of change in the kinetic constant (∂k) and the median value of the kinetic constant (k) normalized by the Anammox population (X_{ANA}) corresponding to k (Equation 2) for each grid (Amer & Kim, 2022; Mozumder, Goormachtigh, et al., 2014). The finite difference method was used to discretize the rate of change in the Anammox population with respect to the rate of change in the kinetic constant (Equation 3) (Chapra & Canale, 1998). The single-factor RSA generally provided valuable insights into the influence of individual kinetic constants on the Anammox population considering the partnership between X_{ANA} and X_{AOB} and the microbial competition between X_{ANA}

TABLE 1 Model parameters and calibration targets at T = 20°C and pH = 7.0.

Model parameter	Symbol	Study A	Study B	Study C	Baseline of this study	Reference
Heterotrophic bacteria (X_H)						
Maximum specific growth rate (1/d)	μ_H	6.0	6.0	6.0	6.0	Henze et al., 2000
Maximum endogenous respiration rate (1/d)	b_H	0.4	0.4	0.4	0.4	Henze et al., 2000
Anoxic reduction factor for μ_H (−)	η_d	0.8	0.8	0.8	0.8	Henze et al., 2000
Oxygen saturation constant (mg-O_2/L)	$K_{O_2}^{H}$	0.2	0.2	0.2	0.2	Henze et al., 2000
Substrate saturation constant (mg-COD/L)	K_{COD}^{H}	20	20	20	20	Henze et al., 2000
Ammonium saturation constant (mg-N/L)	$K_{NH_4}^{H}$	0.05	0.05	0.05	0.05	Henze et al., 2000
Nitrite saturation constant (mg-N/L)	$K_{NO_2}^{H}$	0.5	0.5	0.5	0.5	Henze et al., 2000
Nitrate saturation constant (mg-N/L)	$K_{NO_3}^{H}$	0.5	0.5	0.5	0.5	Henze et al., 2000
Ammonia oxidizing bacteria (X_{AOB})						
Maximum specific growth rate (1/d)	μ_{AOB}	2.05	2.05	2.05	2.05	Wiesmann, 1994
Maximum endogenous respiration rate (1/d)	b_{AOB}	0.13	0.13	0.13	0.13	Wiesmann, 1994
Oxygen saturation constant (mg-O_2/L)	$K_{O_2}^{AOB}$	0.6	0.6	0.6	0.6	Wiesmann, 1994
Ammonium saturation constant (mg-N/L)	$K_{NH_4}^{AOB}$	2.4	2.4	2.4	2.4	Wiesmann, 1994
Nitrite oxidizing bacteria (X_{NOB})						
Maximum specific growth rate (1/d)	μ_{NOB}	1.45	1.45	1.45	1.45	Wiesmann, 1994
Maximum endogenous respiration rate (1/d)	b_{NOB}	0.06	0.06	0.06	0.06	Wiesmann, 1994
Oxygen saturation constant (mg-O_2/L)	$K_{O_2}^{NOB}$	1.0	1.0	1.0	1.0	Moussa et al., 2005
Ammonium saturation constant (mg-N/L)	$K_{NH_4}^{NOB}$	0.20	0.20	0.20	0.20	Ma et al., 2017
Nitrite saturation constant (mg-N/L)	$K_{NO_2}^{NOB}$	0.50	0.50	0.50	0.50	Volcke et al., 2010
Anammox bacteria (X_{ANA})						
Maximum specific growth rate (1/d)	μ_{ANA}	0.10	0.033	0.08	0.10	This study.
Maximum endogenous respiration rate (1/d)	b_{ANA}	0.003	0.003	0.003	0.003	This study.
Oxygen saturation constant (mg-O_2/L)	$K_{O_2}^{ANA}$	0.10	0.10	0.10	0.10	This study.
Ammonium saturation constant (mg-N/L)	$K_{NH_4}^{ANA}$	0.07	0.07	0.07	0.07	This study.
Nitrite saturation constant (mg-N/L)	$K_{NO_2}^{ANA}$	0.05	0.05	0.05	0.05	This study.
Hydrolysis						
Hydrolysis rate constant (1/d)	q_H	3	3	3	3	Henze et al., 2000
Saturation constant for particulate COD (g-X_S/g-X_H)	K_X	0.1	0.1	0.1	0.1	Henze et al., 2000
Anoxic reduction for q_H (−)	η_H	0.6	0.6	0.6	0.6	Henze et al., 2000
Stoichiometric parameters						
Yield of X_H on substrate (g-COD/g-COD)	Y_H	0.63	0.63	0.63	0.63	Henze et al., 2000
Yield of X_{AOB} on ammonium (g-COD/g-N)	Y_{AOB}	0.15	0.15	0.15	0.15	Wiesmann, 1994
Yield of X_{NOB} on nitrite (g-COD/g-N)	Y_{NOB}	0.041	0.041	0.041	0.041	Wiesmann, 1994
Yield of X_{ANA} on nitrite (g-COD/g-N)	Y_{ANA}	0.159	0.159	0.159	0.159	Lackner et al., 2008
Nitrogen content in biomass (g-N/g-COD)	i_{NBM}	0.07	0.07	0.07	0.07	Henze et al., 2000
Inert content in lysis biomass (g-COD/g-COD)	f_i	0.1	0.1	0.1	0.1	Henze et al., 2000

TABLE 2 Summary of simulation conditions and calibration targets of the calibrated model and previous Anammox studies.

Figure	Figure 1	Figure S1	Figure S2	Figure 5
Reference	Corbalá-Robles et al., 2016	Liu, Niu, et al., 2017	Hao et al., 2002	Baseline of this study
S_{NH_4} in the bulk solution (mg-N/L)	1850 ± 100	50	80	500
S_{NO_2} in the bulk solution (mg-N/L)	N/A	N/A	0	10
S_{NO_3} in the bulk solution (mg-N/L)	N/A	N/A	0	10
S_{COD} in the bulk solution (mg-COD/L)	600 ± 30	20	0	0
S_{O_2} in the bulk solution (mg-O_2/L)	0.25	0.3	0.6	0.10
Granule diameter/biofilm thickness (μm)	1100	600	700	1000
Liquid film thickness (μm)	100	100	100	200
Diffusivity of S_{COD} in biofilm × 10^{-4} (m^2/day)	0.15	0.35	0.10	0.15
Diffusivity of S_{O2} in biofilm × 10^{-4} (m^2/day)	0.60	1.45	0.30	0.60
Diffusivity of S_{NH4} in biofilm × 10^{-4} (m^2/day)	0.65	1.35	0.10	0.55
Diffusivity of S_{NO2} in biofilm × 10^{-4} (m^2/day)	0.60	1.30	0.05	0.50
Diffusivity of S_{NO3} in biofilm × 10^{-4} (m^2/day)	0.40	1.20	0.03	0.50
Reaction media	Granule	Granule	Biofilm	Granule

and X_{NOB}. The sensitivity analysis focused on single-factor variations since the model structure inherently includes process rates and biological reactions that capture predictable and independent effects of microbial kinetic constants on different microbial communities.

$$f_{RS} = \frac{\sum_{r=0}^{r=n} \frac{\partial X_{ANA}(r)}{\partial k} \cdot \frac{k}{X_{ANA}(r)}}{n} \quad (2)$$

$$\frac{\partial X_{ANA}(r)}{\partial k} = \frac{X_{ANA}(r, k+\Delta k) - X_{ANA}(r, k-\Delta k)}{2\Delta k} \quad (3)$$

where n is the number of discretized grids in r direction ($n = 20$ grids) and Δ is the change in the median of a kinetic constant ($\Delta = 1\%$).

RESULTS AND DISCUSSION

Model calibration with literature simulation results

The kinetic constants of Anammox bacteria, liquid film thickness (i.e., mixing conditions), and diffusion coefficient of soluble components were determined by calibrating the mathematical model using the simulation results of previous Anammox studies (Corbalá-Robles et al., 2016; Hao et al., 2002; Liu, Niu, et al., 2017) (Figure 1, S1, S2, and Table 2). The calibrated maximum specific growth rate constant of X_{ANA} (μ_{ANA}) ranged from 0.033 to 0.10 d^{-1} while the Anammox bacteria decay rate constant (b_{ANA}) was 0.003 d^{-1}, oxygen half-saturation constant of X_{ANA} ($K_{O_2}^{ANA}$) was 0.10 mg-O_2/L, ammonia half-saturation constant ($K_{NH_4}^{ANA}$) was 0.07 mg-N/L and nitrite half-saturation constant ($K_{NO_2}^{ANA}$) was 0.05 mg-N/L. The determined kinetic constant ranges of X_{ANA} are consistent with those mentioned or estimated in previous Anammox studies (Bi et al., 2015; Corbalá-Robles et al., 2016; Elsayed et al., 2022; Hao et al., 2002; Koch et al., 2000; Lackner et al., 2008; Liu et al., 2020; Liu, Wang, et al., 2021; Mozumder, Goormachtigh, et al., 2014; Ni et al., 2013; Ni et al., 2014; Strous et al., 1998; Volcke et al., 2010; Zhang et al., 2017) (Table 3). In the previous Anammox studies, there was a high variability of μ_{ANA} because it is highly affected by multiple factors such as Anammox species, biomass type, and operating temperature (Table 3) (Oshiki et al., 2011; Zhang et al., 2017). On the other hand, the variability in other microbial kinetic constants of Anammox (e.g., b_{ANA} and $K_{NH_4}^{ANA}$) was negligible (Table 3). For example, most of the previous Anammox studies estimated and/or mentioned that b_{ANA} is ranged between 0.0026 and 0.003 d^{-1} while the range of $K_{NH_4}^{ANA}$ is 0.03–0.07 mg-N/L (Corbalá-Robles et al., 2016; Elsayed et al., 2022; Hao et al., 2002; Lackner et al., 2008; Ni et al., 2014; Volcke et al., 2010).

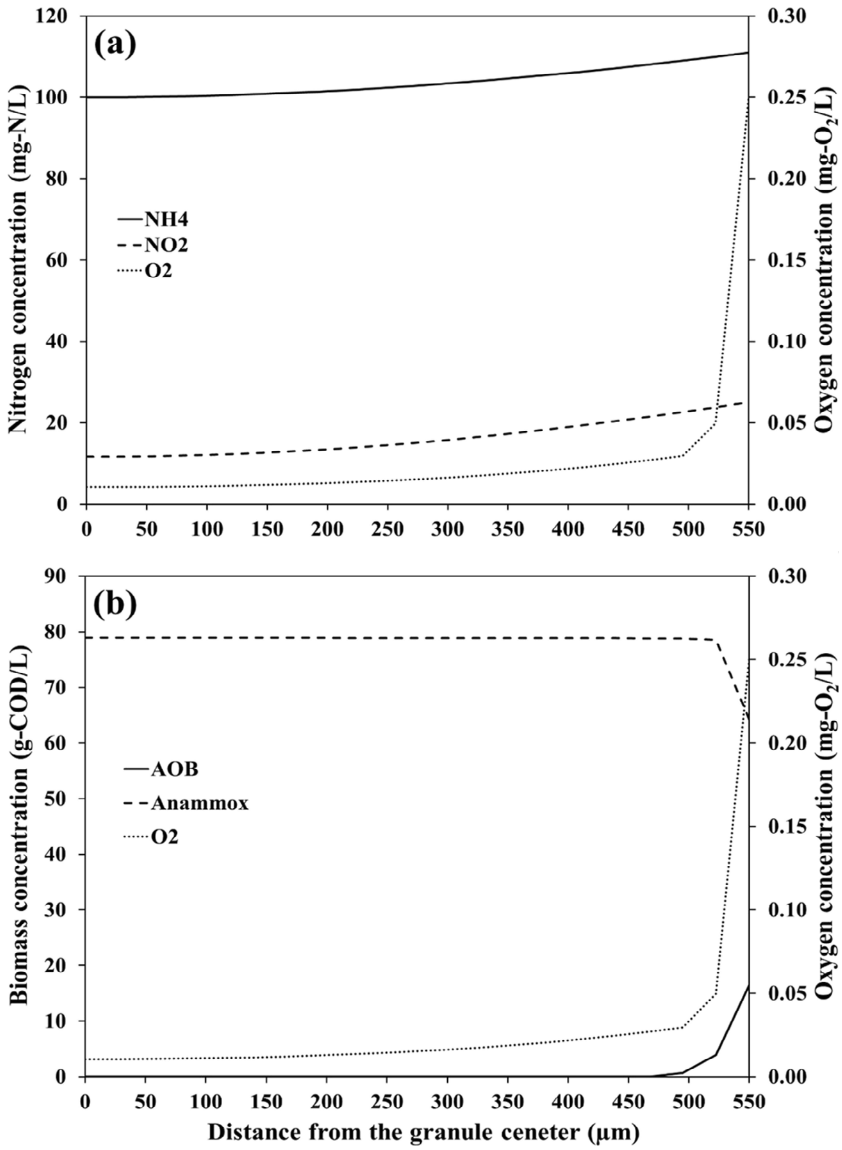


FIGURE 1 Simulation results of the model calibration using study A (Corbalá-Robles et al., 2016) for the concentration profiles of: (a) soluble components (i.e., ammonia, nitrite, and oxygen) and (b) particulate components (i.e., X_{AOB},X_{ANA}) and oxygen concentration profile (simulation conditions are summarized in Tables 1 and 2).

The calibrated liquid film thickness (approximately 100 μm) is also consistent with the assumed values in previous mathematical model studies (Bishop et al., 1997; Li, Du, et al., 2018; Martin et al., 2017; Matsumoto et al., 2007; Wäsche et al., 2002) (Table 3). The reported values of liquid film thickness in previous studies ranged from 50 to 300 μm where a 100-μm thick liquid film was commonly adopted in the modeling of nitrogen removal systems (Li, Du, et al., 2018; Martin et al., 2017; Matsumoto et al., 2007). Based on the mathematical model calibration, the diffusion coefficient of soluble components were estimated to be 0.10–0.35 × 10^{-4} m^2/day for S_{COD}, 0.30–1.45 × 10^{-4} m^2/day for S_{O_2}, 0.10–1.35 × 10^{-4} m^2/day for S_{NH_4}, 0.05–1.30 × 10^{-4} m^2/day for S_{NO_2} and 0.03–1.20 × 10^{-4} m^2/day for S_{NO_3}. The determined diffusion coefficients are comparable with the ranges of the coefficients used or mentioned in previous biofilm model studies (Elsayed et al., 2021; Stewart, 1998, 2003) (Table 3). It should be highlighted that the diffusivity of each substrate is highly impacted by the molecule size, structure, and charge (Stewart, 2003). For example, the diffusivity of COD from the bulk solution into the granules was the least compared to the rest of the soluble components (Table 3) because it has the highest molecular weight and most complicated structure which increases the resistance against particle diffusion into the granules and biofilms. On the other hand, oxygen can be easily diffused through the liquid film into the granules since it is a non-polar gas which allows quick penetration into the microbial granules.

The mathematical model successfully reproduced the simulation results of the three previous Anammox studies (Corbalá-Robles et al., 2016; Hao et al., 2002; Liu, Niu, et al., 2017) (Figure 1, S1, S2). The model accurately captured microbial stratification and substrate diffusion within the granules which are consistent with the

TABLE 3 Summary of the estimated calibration parameters in this study and reported values in previous studies.

Parameter	Estimated value in this study	Reported value in previous studies	Reference
Anammox maximum specific growth rate (μ_{ANA}) (1/d)	0.033–0.10	0.08–0.09	Koch et al., 2000
		0.072	Hao et al., 2002
		0.08	Lackner et al., 2008
		0.052	Volcke et al., 2010
		0.053	Ni et al., 2014
		0.028–0.072	Bi et al., 2015
		0.052	Corbalá-Robles et al., 2016
		0.11–0.33	Zhang et al., 2017
		0.048–0.10	Elsayed et al., 2022
Anammox maximum endogenous respiration rate (b_{ANA}) (1/d)	0.003	0.003	Hao et al., 2002
		0.003	Lackner et al., 2008
		0.0026	Volcke et al., 2010
		0.003	Ni et al., 2014
		0.00016–0.003	Bi et al., 2015
		0.0026	Corbalá-Robles et al., 2016
		0.003	Elsayed et al., 2022
Anammox oxygen saturation constant ($K_{O_2}^{ANA}$) (mg-O_2/L)	0.10	0.40	Koch et al., 2000
		0.01	Hao et al., 2002
		0.01	Lackner et al., 2008
		0.01	Volcke et al., 2010
		0.01	Ni et al., 2014
		0.01–0.40	Bi et al., 2015
		0.10	Elsayed et al., 2022
Anammox ammonium saturation constant ($K_{NH_4}^{ANA}$) (mg-N/L)	0.07	0.07	Hao et al., 2002
		0.07	Lackner et al., 2008
		0.03	Volcke et al., 2010
		0.07	Ni et al., 2014
		0.07–0.73	Bi et al., 2015
		0.03	Corbalá-Robles et al., 2016
		0.07	Elsayed et al., 2022
Anammox nitrite saturation constant ($K_{NO_2}^{ANA}$) (mg-N/L)	0.05	0.05	Lackner et al., 2008
		0.005	Volcke et al., 2010
		0.05–5	Ni et al., 2013
		0.05	Ni et al., 2014
		0.035–0.55	Bi et al., 2015
		0.005	Corbalá-Robles et al., 2016
		0.05	Elsayed et al., 2022
Liquid film thickness (L_f) (μm)	100	50–300	Wäsche et al., 2002
		100	

TABLE 3 (Continued)

Parameter	Estimated value in this study	Reported value in previous studies	Reference
			Matsumoto et al., 2007
		100	Martin et al., 2017
		100	Li, Du, et al., 2018
		100–250	Elsayed et al., 2021
Diffusivity of S_{COD} in biofilm × 10^{-4} (m^2/day)	0.10–0.35	0.04–0.49	Stewart, 2003
		0.10–0.18	Elsayed et al., 2021
Diffusivity of S_{O2} in biofilm × 10^{-4} (m^2/day)	0.30–1.45	0.44–1.36	Stewart, 2003
		0.45–0.65	Elsayed et al., 2021
Diffusivity of S_{NH4} in biofilm × 10^{-4} (m^2/day)	0.10–1.35	0.57–1.38	Stewart, 2003
		0.40–0.75	Elsayed et al., 2021
Diffusivity of S_{NO2} in biofilm × 10^{-4} (m^2/day)	0.05–1.30	0.56–1.35	Stewart, 2003
		0.40–0.75	Elsayed et al., 2021
Diffusivity of S_{NO3} in biofilm × 10^{-4} (m^2/day)	0.03–1.20	0.55–1.34	Stewart, 2003
		0.40–0.75	Elsayed et al., 2021

simulation results of the previously mentioned Anammox studies. The consistency of these results with those of previous Anammox studies reinforces the reliability of the model in replicating the spatial distribution of microbial communities and substrate utilization in single-stage PN/A systems.

Effect of dissolved oxygen on microbial communities

A high dissolved oxygen concentration in the bulk solution, typically defined as DO $\geq$ 0.50 mg-O_2/L in the Anammox operation process (Cao et al., 2017; Lackner et al., 2014; Lotti et al., 2015; Yin et al., 2016), increased the nitrifier population (X_{AOB} and X_{NOB}) at the outer side of granules (Figure 2a and b). On the other hand, such elevated concentrations of dissolved oxygen inhibited the growth of X_{ANA} (Figure 2c) because the nitrite produced by X_{AOB} was completely consumed by X_{NOB} under aerobic conditions at the outer layer of granules. This finding is consistent with the results of previous Anammox studies (Elsayed et al., 2022; Hoekstra et al., 2019; Liu, Niu, et al., 2017) where it was demonstrated that high oxygen concentration has a negative effect on the growth of X_{ANA} population. Although the X_{ANA} population was hardly affected when the oxygen concentration in the bulk solution increased from 0.50 to 1.0 mg-O_2/L (Figure 2c), the microbial populations of nitrifiers were approximately doubled at the outer layer of granules (Figure 2a and b) where the microbial population increased from 10.3 to 19.7 g-COD/L for X_{AOB} and from 4.3 to 8.6 g-COD/L for X_{NOB}. These findings are consistent with the previously mentioned results in other nitrogen removal studies (Chaali et al., 2018; Landreau et al., 2020; Laureni et al., 2016; Li et al., 2023), confirming the importance of oxygen for a high growth rate of X_{AOB} and X_{NOB}. In addition, the oxygen penetration depth increased with the increase of dissolved oxygen concentration in the bulk solution where it reached 100 μm at dissolved oxygen concentration of 1.0 mg-O_2/L. This penetration depth is comparable with those observed and determined in previous studies (Lv et al., 2022a; Lv et al., 2022b; Morales et al., 2015, 2016; Yuan et al., 2022) where they varied from 80 to 150 μm according to the oxygen concentration.

At extremely low dissolved oxygen concentration (DO ≅ 0.01 mg-O_2/L), there was no microbial activity for X_{AOB} due to the absence of oxygen (Figure 2a), leading to a significant shortage in the amount of nitrite within the granules. This negatively affected the microbial communities of X_{ANA} and X_{NOB} due to the absence of nitrite (Figure 2b and c). These results are comparable with the previously reported findings in other Anammox studies (Jeong et al., 2021; Li et al., 2020; Miao et al., 2017; Val de Rio et al., 2019), emphasizing the partnership between X_{AOB} and X_{ANA}. However, the population of X_{ANA} was still higher than those at relatively high

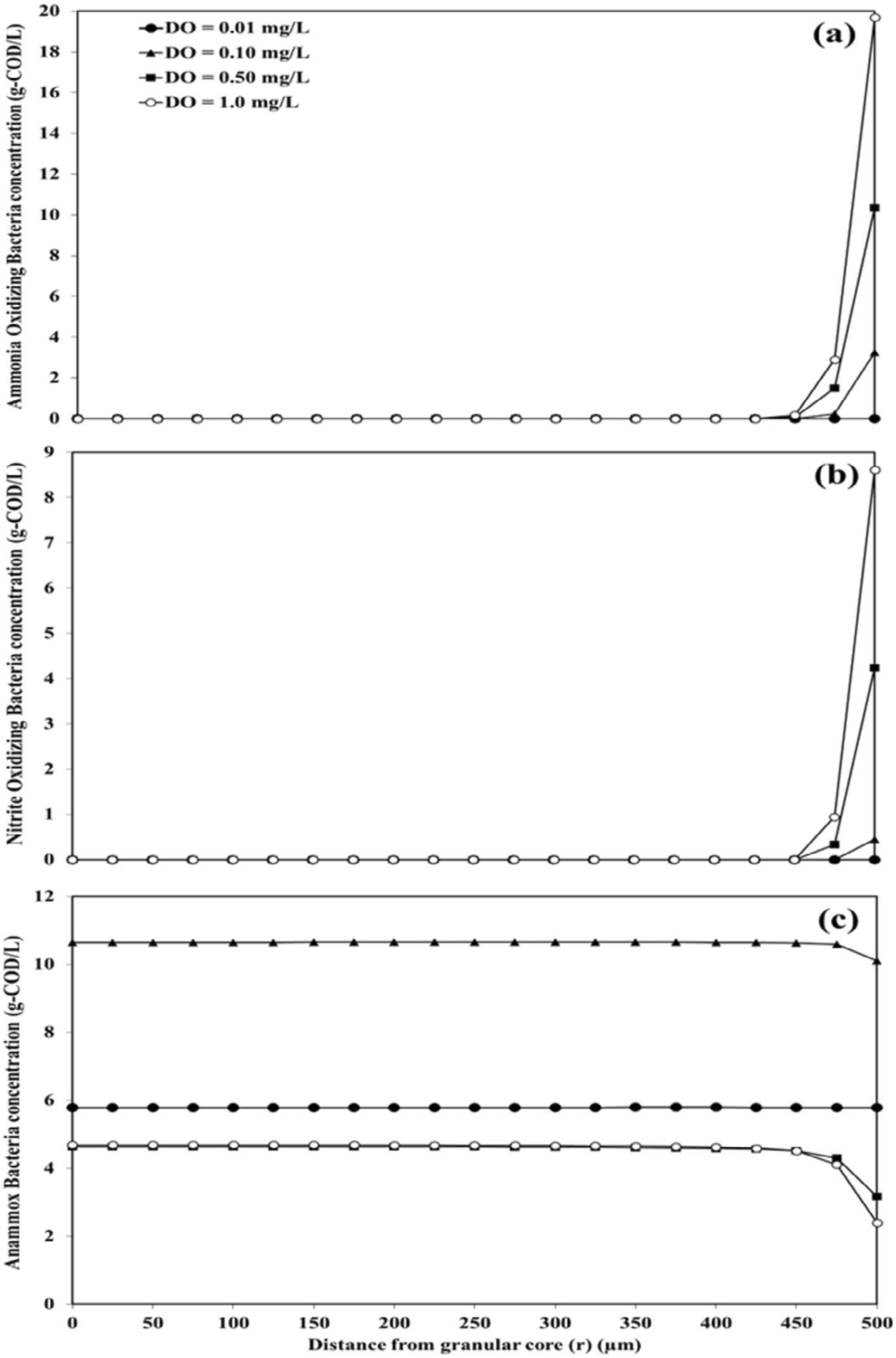


FIGURE 2 Effect of dissolved oxygen concentration in the bulk solution on the population of: (a) ammonia-oxidizing bacteria (X_{AOB}), (b) nitrite-oxidizing bacteria (X_{NOB}), and (c) anaerobic ammonium oxidation bacteria (X_{ANA}). (simulation conditions: liquid film thickness = 200 μm, ammonia concentration = 500 mg-N/L, and COD concentration = 0, other conditions are summarized in Tables 1 and 2).

oxygen concentration (DO ≥ 0.50 mg-O_2/L), confirming the inhibition of high oxygen concentration to the activity of Anammox bacteria in the single-stage PN/A processes.

Based on the simulation results, it was found that moderate dissolved oxygen concentration in the bulk solution (DO = 0.10 mg-O_2/L) is the optimal condition for the growth of X_{ANA} population (Figure 2c) because it allowed the creation of nitrite by X_{AOB}, providing sufficient amount for the growth of X_{ANA} without any competition from X_{NOB} under limited oxygen conditions. These results are consistent with those obtained and observed in other previous Anammox studies (Deng et al., 2021, 2022; Elsayed et al., 2022; Gong et al., 2021; Zhang et al., 2019; Zhao et al., 2023; Zou et al., 2018) where the recommended oxygen concentration varied from 0.10 to 0.40 mg-O_2/L for successful Anammox operation. It was also noticed that the average population of X_{ANA} at moderate oxygen conditions (10.6 g-COD/L) was greater than those at the other simulated oxygen concentrations by approximately two times (Figure 2c). Therefore, it is highly recommended to maintain the oxygen concentration at a relatively moderate level (i.e., DO = 0.10 mg-O_2/L) for successful single-stage PN/A operation, keeping a balance between the nitrite production by X_{AOB} and its utilization by X_{ANA}.

Effect of COD concentration on microbial communities

The decreasing COD concentration enhanced the growth of X_{ANA} population (Figure 3c) because it allowed X_{AOB} to consume oxygen without any competition from the heterotrophic bacteria (Figure 3a and d), producing enough nitrite for efficient Anammox growth in the single-stage PN/A processes under limited oxygen conditions. This result is consistent with the findings and observations of previous Anammox studies (Hu et al., 2023; Kang et al., 2018; Wang et al., 2022) where it was demonstrated that low COD concentration enhanced the operation of Anammox technology. At extremely low COD concentration (COD ≅ 0), there was no activity for the heterotrophic bacteria (Figure 3d), providing favorable conditions for X_{AOB} to utilize oxygen at the outer layer of granules (Figure 3a and b). This finding is consistent with the previously reported results in other studies (Al-Hazmi et al., 2023; Trinh et al., 2021; Zhang et al., 2022), highlighting the role of the competition between the nitrifiers and heterotrophs in consuming oxygen. In some cases, extracellular polymeric substances (EPS) and microbial metabolites can act as an internal source of COD for heterotrophic bacteria. However, in the current study, the contribution of these substrates was negligible at extremely low COD concentrations (COD ≅ 0) where the microbial activity of heterotrophs was minimal (Figure 3d). This suggests that the bioavailable fraction of EPS and microbial metabolites was insufficient to provide COD to sustain significant heterotrophic growth. This observation is consistent with the main findings of previous studies emphasizing that the production of EPS and microbial metabolites is often limited under autotrophic conditions when there is no external organic carbon (Guerriero et al., 2022; Kang

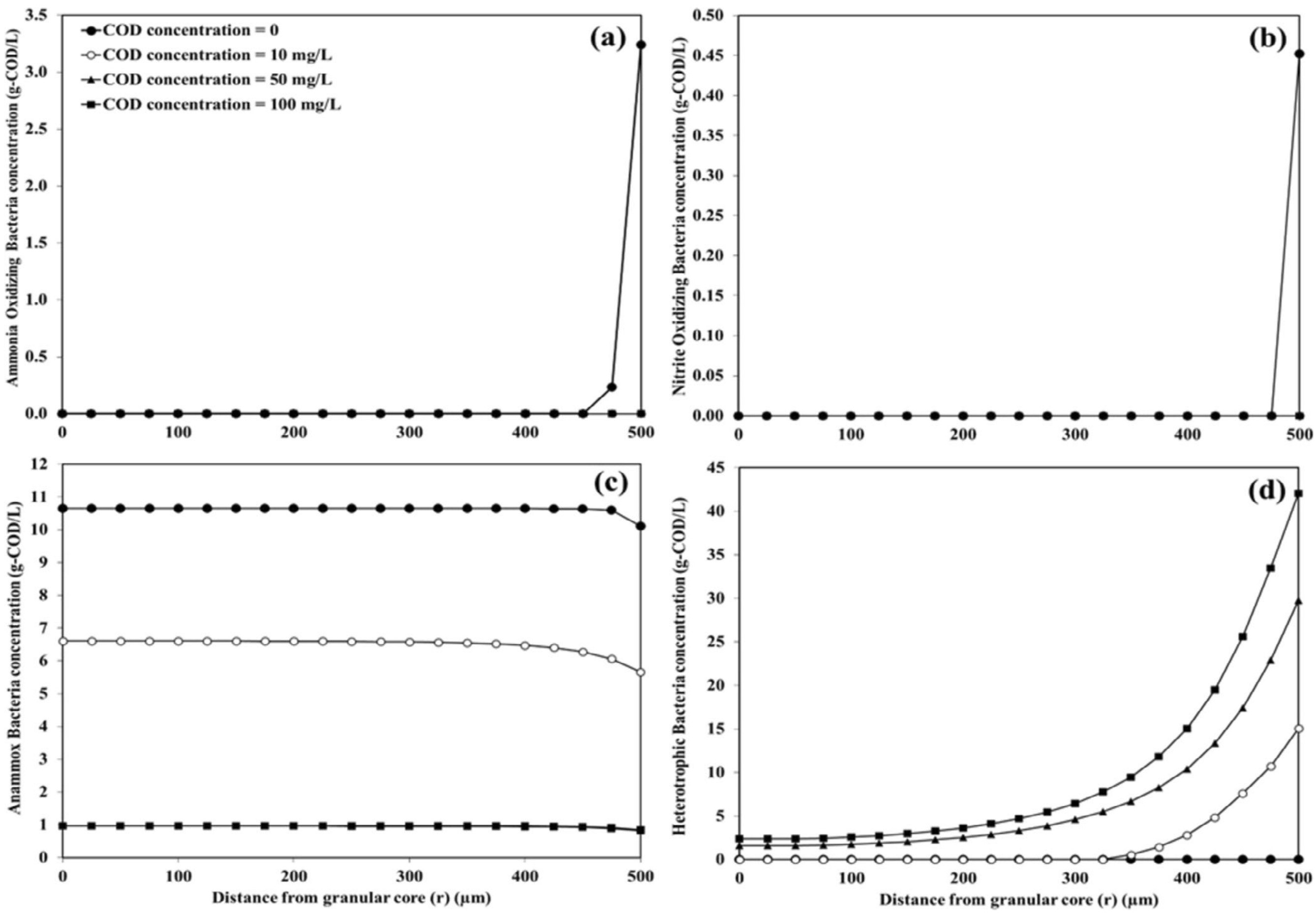


FIGURE 3 Effect of COD concentration on the population of: (a) ammonia-oxidizing bacteria (X_{AOB}), (b) nitrite-oxidizing bacteria (X_{NOB}), (c) anaerobic ammonium oxidation bacteria (X_{ANA}), and (d) heterotrophic bacteria (simulation conditions: liquid film thickness = 200 μm, ammonia concentration = 500 mg-N/L, and DO concentration = 0.10 mg-O_2/L, other conditions are summarized in Tables 1 and 2).

et al., 2014; Wang et al., 2023). Therefore, it is highly recommended to maintain a minimum COD concentration in the bulk solution for effective single-stage PN/A processes.

For higher concentrations of COD (COD ≥ 10 mg-COD/L) and moderate dissolved oxygen concentration in the bulk solution (DO = 0.10 mg-O_2/L), there was no microbial activity of X_{AOB} and X_{NOB} while the heterotrophic bacteria were dominant at the outer layer of granules because heterotrophs have greater maximum specific growth rate constant ($K_{O_2}^{H}$) and lower oxygen half-saturation constant (μ_H) compared to X_{AOB} and X_{NOB} (Table 1). The domination of heterotrophs and inactivity of X_{AOB} negatively affected the growth rate of X_{ANA} population due to the lack of nitrite within the granules. At COD concentration of 10 mg-COD/L, the average population of Anammox bacteria decreased by 40% compared to those at extremely low COD concentration (COD ≅ 0). It should be also noted that the average population of Anammox bacteria decreased by 90% compared to those at extremely low COD concentration when the COD concentration in the bulk solution was higher than or equal to 50 mg-COD/L (Figure 3c), reflecting the negative impact of increasing COD concentration on the operation of single-stage PN/A technology. On the other hand, the microbial growth of the heterotrophic bacteria was enhanced with the increase of COD concentration where the population of the heterotrophs at the outer part of granules increased by approximately 50% when the COD concentration increased from 50 to 100 mg-COD/L because of the substrate availability.

Effect of liquid film thickness on microbial communities

Thin liquid films (i.e., boundary layers), reflecting turbulent and good mixing conditions in the bulk solution, enhanced the growth of X_{AOB} and X_{NOB} at the outer layer of granules (Figure 4a and b) because thin layers allowed better diffusion of ammonia and oxygen from the bulk side to granules. On the other hand, the population of X_{ANA} was decreased during turbulent mixing conditions (Figure 4c) as rapid diffusion of oxygen through the thin boundary layer allowed higher utilization of nitrite by X_{NOB}, inhibiting the activity of X_{ANA}. In contrast, thick liquid films enhanced the growth of X_{ANA} population compared to relatively thin films because thick layers acted as a barrier for rapid diffusion of oxygen, providing favorable conditions for better microbial activity of X_{ANA} under limited oxygen conditions. At liquid film thickness ≥ 200 μm, the average population of Anammox bacteria was approximately doubled compared to the population at turbulent mixing conditions ($L_f = 0$) (Figure 4c), indicating the importance of considering mixing conditions in a successful Anammox operation. On the other hand, the activity of nitrifiers (i.e., X_{AOB} and X_{NOB}) decreased in poor mixing condition (i.e., thick liquid films) due to the slow diffusion of oxygen through the boundary layer. Based on the simulation results, it was concluded that moderate liquid films (i.e., $L_f = 100$ μm) are optimal for effective single-stage PN/A processes where the average X_{ANA} population reached 12.1 g-COD/L (15% higher than thicker layers) (Figure 4c). Therefore, it is highly recommended to maintain moderate mixing conditions to balance the microbial activity of X_{AOB} and X_{ANA} in single-stage PN/A operation.

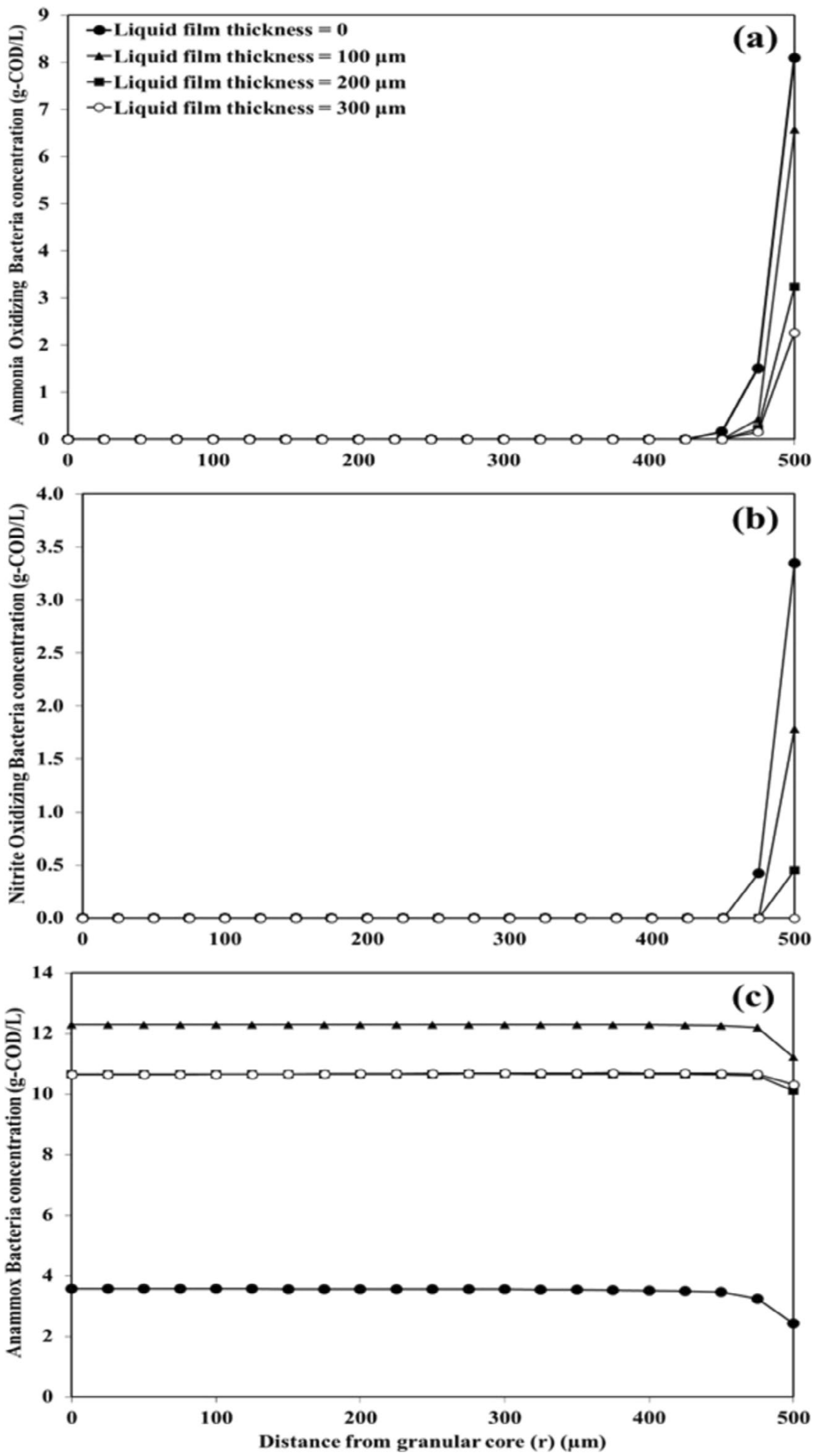
Effect of X_{ANA} kinetics on Anammox population

Based on the results of the single-factor RSA on the kinetic constants of X_{ANA}, it was found that μ_{ANA} is a governing parameter on the growth of X_{ANA} population while the X_{ANA} population was not significantly affected by the decay rate constant (b_{ANA}) (Figure 5). This model simulation result is consistent with the results of other Anammox studies (Pérez et al., 2014; Zhang et al., 2017), reflecting the importance of μ_{ANA} on efficient single-stage PN/A processes. The Anammox population was positively correlated with μ_{ANA} because high μ_{ANA} enhanced the ammonia and nitrite utilization by X_{ANA}, resulting in better operation of single-stage PN/A process. It was also noticed that the Anammox population was positively correlated with the oxygen half-saturation constant of X_{ANA} ($K_{O_2}^{ANA}$) (Figure 5) because high $K_{O_2}^{ANA}$ resulted in less inhibition to Anammox bacteria by oxygen. On the other hand, the Anammox population and b_{ANA} were in a negative correlation as high b_{ANA} caused high lysis rate of Anammox bacterial cells, resulting in insufficient growth of X_{ANA} population in the single-stage PN/A system.

Partnership between X_{ANA} and X_{AOB}

For the kinetic constants of X_{AOB}, the maximum specific growth rate constant of X_{AOB} (μ_{AOB}) was positively correlated with X_{ANA} population (Figure 5) because high μ_{AOB} under moderate dissolved oxygen concentration (DO = 0.10 mg-O_2/L) increased the creation rate of nitrite at the outer layer of granules, providing sufficient amount of substrate for efficient operation of the single-stage PN/A processes. Also, μ_{AOB} controlled the partnership between X_{AOB} and X_{ANA} where X_{ANA} utilized the produced nitrite from aerobic ammonia oxidation

FIGURE 4 Effect of liquid film thickness (i.e., mixing conditions) on the population of: (a) ammonia-oxidizing bacteria (X_{AOB}), (b) nitrite-oxidizing bacteria (X_{NOB}), and (c) anaerobic ammonium oxidation bacteria (X_{ANA}) (simulation conditions: COD concentration = 0, ammonia concentration = 500 mg-N/L, and DO concentration = 0.10 mg-O_2/L, other conditions are summarized in Tables 1 and 2).



by X_{AOB} at the inner layer of granules. It should also be mentioned that although there is a competition between X_{AOB} and X_{ANA} on ammonia in some cases, ammonia was not the rate-limiting substrate in these simulations as ammonia concentration in the bulk solution was 500 mg-N/L under limited oxygen conditions (DO = 0.10 mg-O_2/L) (Table 2). This interpretation is consistent with the results emphasized in previous Anammox studies (Hoekstra et al., 2019; Laureni et al., 2016; Liu et al., 2016; Zhuang et al., 2022), confirming that ammonia concentration is not dominant in the growth of X_{ANA} population at high ammonia concentrations. The oxygen half-saturation constant ($K_{O_2}^{AOB}$) and the decay rate constant (b_{AOB}) of X_{AOB} were negatively correlated with the Anammox population as large $K_{O_2}^{AOB}$ and high lysis rate relatively decreased the activity of X_{AOB},

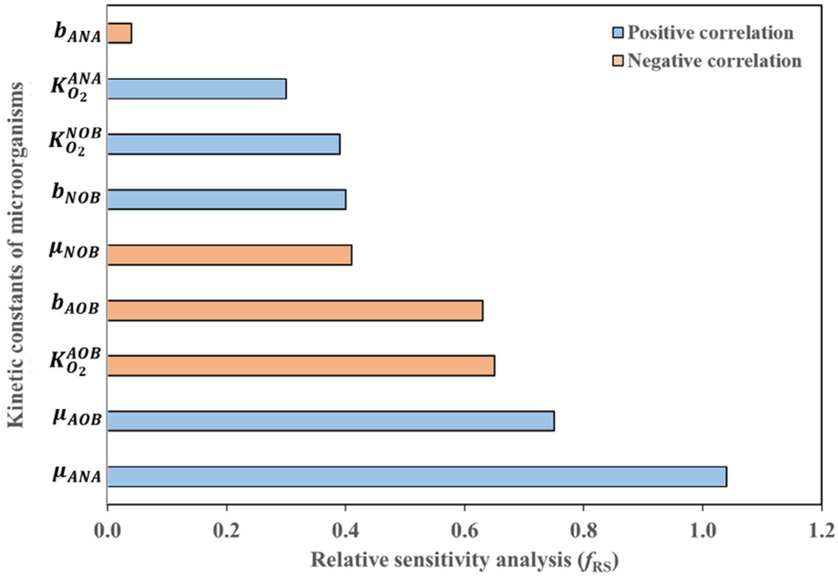


FIGURE 5 Single-factor relative sensitivity analysis (RSA) for the effect of X_{ANA}, X_{AOB}, and X_{NOB} kinetic constants on the Anammox population (X_{ANA}) (simulation conditions: COD concentration = 0, ammonia concentration = 500 mg-N/L, and DO concentration = 0.10 mg-O_2/L, liquid film thickness = 200 µm, other conditions are summarized in Tables 1 and 2).

decreasing the amount of produced nitrite by X_{AOB} which limits the growth of X_{ANA} population due to the absence of nitrite. These findings are consistent with the results obtained and observed in previous Anammox studies (Hu et al., 2023; Wang et al., 2022; Weralupitiya et al., 2021) where the interdependence between X_{AOB} and X_{ANA} was highlighted for efficient Anammox operation process.

Competition between X_{ANA} and X_{NOB}

Based on the results of the single-factor RSA on the kinetics of X_{NOB}, it was found that the maximum specific growth rate constant of X_{NOB} (μ_{NOB}) was negatively correlated with the X_{ANA} population (Figure 5) because low activity of X_{NOB} decreased the competition between X_{ANA} and X_{NOB} on nitrite, providing optimal conditions for successful single-stage PN/A processes. It should also be mentioned that the competition between X_{ANA} and X_{NOB} on nitrite is extremely critical since nitrite is the rate-limiting substrate of active Anammox operation process (Chaali et al., 2018; Elsayed et al., 2022; Ishimoto et al., 2021; Ma et al., 2017; Su et al., 2023). On the other hand, the oxygen half-saturation constant ($K_{O_2}^{NOB}$) and decay rate constant (b_{NOB}) of X_{NOB} were positively correlated with X_{ANA} population (Figure 5) due to the positive influence of inactivity of X_{NOB} on the growth rate of X_{ANA}. These findings are consistent with those found in previous Anammox studies (Cao et al., 2017; Li, Li, et al., 2018; Mozumder, Picioreanu, et al., 2014) where it was demonstrated that controlling the competition between X_{NOB} and X_{ANA} is crucial for healthy Anammox process. As a result, maintaining low activity of X_{NOB} by lowering μ_{NOB} and/or enlarging $K_{O_2}^{NOB}$ and b_{NOB} can result in higher X_{ANA} population within granules, emphasizing that X_{NOB} should be suppressed for better Anammox applications.

CONCLUSIONS

This study successfully developed and calibrated a one-dimensional steady-state mathematical model to characterize the rapid growth of Anammox bacteria in the single-stage PN/A granulation processes. The mathematical model effectively replicated the simulation results of previous Anammox studies to determine the kinetic constants of X_{ANA} growth and mass transport in the granules. The simulation results of the mathematical model revealed that maintaining moderate DO concentration (≅ 0.10 mg-O_2/L) and minimum COD concentration (COD ≅ 0) enhanced the growth of X_{ANA} by controlling their competition with X_{NOB} and heterotrophs. In addition, moderate liquid films (L_f ≅ 100 µm) are preferable to decrease the diffusion of oxygen to deep layers of individual granules, balancing the nitrite production by X_{AOB} and the competition between X_{ANA} and X_{NOB} for nitrite. A single-factor relative sensitivity analysis (RSA) was also performed to determine the role of microbial kinetic constants of X_{AOB}, X_{NOB}, and X_{ANA} on the growth of X_{ANA}. It was found that μ_{ANA} and $K_{O_2}^{ANA}$ had a crucial role in enhancing the growth of X_{ANA}. For the kinetic constants of X_{AOB}, μ_{AOB} was a governing factor on the growth of X_{ANA} population because of the partnership between X_{AOB} and X_{ANA}. On the other hand, high μ_{NOB} negatively affected the growth of X_{ANA} due to the microbial competition between X_{NOB} and X_{ANA}, reflecting the importance of X_{NOB} suppression for efficient operation of single-stage PN/A technology. The results of this study can lead to better understanding of the governing parameters on rapid Anammox growth in the single-stage PN/A processes using granulation. In addition, the main research outcomes can be applied to allow mainstream Anammox treatment at low ammonia concentration (i.e., 50 mg-N/L or lower) using the single-stage PN/A processes.

AUTHOR CONTRIBUTIONS

Ahmed Elsayed: Writing – original draft; investigation; visualization; software; formal analysis; data curation; methodology. **Taeho Lee:** Conceptualization; writing – review and editing; validation; methodology; funding acquisition. **Younggy Kim:** Conceptualization; funding acquisition; writing – review and editing; methodology; project administration; resources; supervision.

ACKNOWLEDGEMENTS

This study was supported by the Ontario Ministry of Research and Innovation (Ontario Research Fund-Research Excellence, RE09-077), Natural Sciences and Engineering Research Council of Canada (Discovery Grants, RGPIN-2019-06747 and Discovery Accelerator Supplement, RGPAS-2019-00102), and National Research Foundation of Korea (Brain Pool Program, 2018H1D3A2065879).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Elsayed, A., Lee, T., & Kim, Y. (2025). Maximizing the efficiency of single-stage partial nitrification/Anammox granule processes and balancing microbial competition using insights of a numerical model study. *Water Environment Research*, *97*(3), e70059. https://doi.org/10.1002/wer.70059