Journal of Korean Society on Water Environment, Vol. 38, No. 4, pp. 171-176 (July, 2022)
pISSN 2289-0971 eISSN 2289-098X https://doi.org/10.15681/KSWE.2022.38.4.171

2중 구조의 PVA/alginate 겔 비드에서의 독립영양 단일공정 질소제거효율 시뮬레이션

배효관†

부산대학교 사회환경시스템공학과

Simulated Nitrogen Removal for Double-Layered PVA/Alginate Structure for Autotrophic Single-Stage Nitrogen Removal

Hyokwon Bae†

Department of Civil and Environmental Engineering, Pusan National University

(Received 24 November 2021, Revised 21 July 2022, Accepted 27 July 2022)

Abstract

Recently, an autotrophic single-stage nitrogen removal (ASSNR) process based on the anaerobic ammonium oxidation (ANAMMOX) reaction has been proven as an economical ammonia treatment. It is highly evident that double-layered gel beads are a promising alternative to the natural biofilm for ASSNR because of the high mechanical strength of poly(vinyl alcohol) (PVA)/alginate structure and efficient protection of ANAMMOX bacteria from dissolved oxygen (DO) due to the thick outer layer. However, the thick outer layer results in severe mass transport limitation and consequent lowered bacterial activity. Therefore, the effects of the thickness of the outer layer on the overall reaction rate were tested in the biofilm model using AQUASIM for ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and ANAMMOX bacteria. A thickness of 0.5~1.0 mm is preferred for the maximum total nitrogen (TN) removal. In addition, a DO of 0.5 mg/L resulted in the best total nitrogen removal. A higher DO induces NOB activity and consequent lower TN removal efficiency. The optimal density of AOB and NOB density was 1~10% for a 10% ANAMMOX bacterial in the double-layered PVA/alginate gel beads. The real effects of operating parameters of the thickness of the outer layer, DO and concentrations of biomass balance should be intensively investigated in the controlled experiments in batch and continuous modes.

Key words : AQUASIM, Autotrophic single-stage nitrogen removal, Biomass, Dissolved oxygen, Double-layered gel bead, Simulation, Thickness

---

Corresponding author, 부교수(Assistant Professor), hyokwan.bae@pusan.ac.kr, https://orcid.org/0000-0002-2422-9411

1. Introduction

The functional stability of ammonia-oxidation is a serious design challenge for autotrophic single-stage nitrogen removal (ASSNR) because ammonia-oxidation is correlated to the production of NO_2^--N for anaerobic ammonium oxidation (ANAMMOX) reaction and the protection of ANAMMOX bacteria from the oxygen inhibition. The fabrication method of double-layered gel beads was developed in the previous study (Bae et al., 2017). The core bead, which was fabricated using poly (vinyl alcohol) (PVA) crosslinking reaction, exhibits the core reaction of ANAMMOX to produce nitrogen gas (Bae et al., 2015). Besides, the outer layer, which was constructed based on interfacial crosslinking of PVA with boric acid, lead to the rate-limiting partial-nitritation reaction (Minh et al., 2021). Both the core bead and outer layer include inoculum ANAMMOX and nitrifying bacteria before the crosslinking reaction to entrap a high concentration of inoculum instead of building biofilm. The applicability of the double-layered gel bead for ASSAR was verified in aerobic batch and continuous bioreactors with synthetic ammonia wastewater (Bae et al., 2017). However, the destruction of the outer layer eventually results in the loss of AOB biomass in the ASSAR process. Besides the mechanical strength, special concerns should be taken to the oxygen penetration for the operational stability of ASSNR. DO is the main design factor for biological wastewater treatment processes because of the low solubility of oxygen and the high cost of aeration. A high concentration of DO is the driving force for the oxygen penetration into an activated sludge floc or an immobilization system. As a result, the DO concentration in a bulk phase determines the vertical distribution of oxygen.

Intensified aeration can enhance the ammonia-oxidation reaction, which is the rate-limiting step of ASSNR, but the deep penetration of oxygen through the core beads may lead to the inhibition of ANAMMOX activity. Thus, the oxygen penetration depth controls both activities of ammonia-oxidizing bacteria (AOB) and ANAMMOX bacteria. The characterization of the DO penetration depending on the environmental conditions provides the fundamental information to optimize the operational conditions for ASSNR using the PVA/alginate gel beads. In this study, the DO diffusion was simulated according to operational parameters.

The conventional study to investigate the biofilm structure for biological nitrogen removal has been focused on natural biofilm formation. For example, the resultant nitrogen removal efficiency of the biological nitrogen removal process has been assessed in response to the biofilm thickness (Piculell et al., 2016). The major parameters for these studies were carbon to nitrogen ratio, surface loading of oxygen and substrate concentrations (Matsumoto et al., 2007). In comparison to these previous studies, this study intensively investigates the effects of the fixed thickness of the outer layer which is steady during the continuous process owing to high mechanical strength. The controllable thickness of the outer layer according to the fabrication conditions using PVA concentration and reaction period of the outer layer is one of the attractive advantages of double-layered gel beads. To our best knowledge, the nitrogen removal efficiency of ASSNR in response to the thickness of the PVA gel layer conducting partial nitritation is first simulated in this study. Diffusion of DO through the outer layer is terminated when the DO is entirely depleted by the ammonia-oxidation activity. Thus, the penetration depth is significantly related to the activity of AOB. In this sense, inhibitory factors for AOB, such as free ammonia (FA), free nitric acid (FNA) and toxic chemicals increase the vertical range of oxygen penetration through the outer layer (Kim et al., 2008).

It is highly evident that double-layered gel beads is a promising alternative to the natural biofilm for ASSNR because of the high mechanical strength of PVA/alginate structure and the efficient protection of ANAMMOX bacteria from oxygen due to the thick outer layer. However, the thick outer layer can result in the severe mass transport limitation and consequent lowered bacterial activity. Therefore, the effects of thickness of the outer layer on the overall reaction rate were tested using a biofilm model of AQUASIM in this study. In addition, the bacterial activities including AOB, nitrite-oxidizing bacteria (NOB) and ANAMMOX bacteria were simulated with various DO concentrations.

AQUASIM is a simulation program used to describe an one dimensional multispecies and multisubstrate biofilm system (Wanner et al., 1995). The program is based on the extended mixed culture biofilm considering mass balance equations of particulate and dissolved components that includes biofilm growth and other processes such as attachment and detachment (Wanner and Morgenroth, 2004). AQUASIM effectively estimates various parameters which requires intensive costs and labor to be observed in experiments. For example, distributions of NH_4^+, NO_2^- and NO_3^- though the outer layer can be simulated.

The balance of the three population also significantly affects the nitrogen removal performance because an excessive NOB activity lose the electron acceptor of nitrite for ANAMMOX bacteria. Also, excessive AOB activity lose the electron donor of ammonium for ANAMMOX bacteria. In addition, less amount of ANAMMOX bacteria reduce the total nitrogen (TN) removal efficiency. Thus, the effects of the balance between biomass concentrations of AOB, NOB and ANAMMOX bacteria were simulated through the biofilm

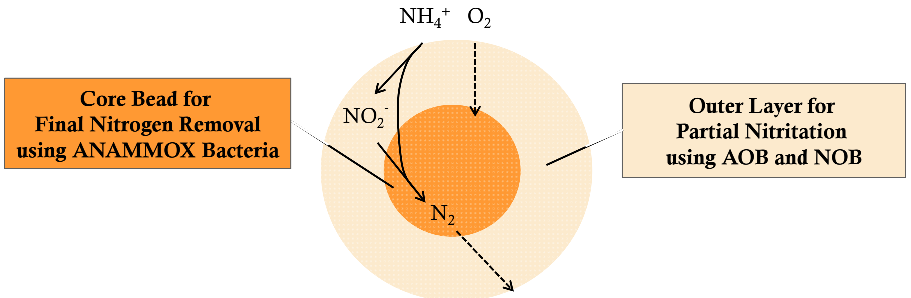

Fig. 1. The structure of the double layered gel bead for autotrophic single-stage nitrogen removal.

model simulation of AQUASIM.

2. Materials and Methods

2.1 Systematic evaluation of ASSNR using AQUSIM

The general structure of the double-layered gel bead for ASSNR is present in Fig. 1. The present investigation using AQUASIM conducts a sensitivity analysis with the aim of parameter optimization for ASSNR using double-layered gel beads. The model parameters for the ASSNR biofilm were obtained from the previous study (Capuno, 2007). The model description for the double-layers possessing AOB, NOB and ANAMMOX bacteria used in the AQUASIM software is summarized in Table 1. Table 2 shows the kinetic parameters

Table 1. The modeled biofilm structure for the double-layered gel beads

Component	Reactions	Bulk Volume	Dimension of biofilm	Biofilm density	Relative density
Aerator for DO control	Increase in DO (Imaginary tank containing target DO)	8,000 L	-	-	-
Link 1	O_2 diffusion	-	-	-	-
Outer layer	Growth and decay of AOB and NOB	0.5 L	0.16 m^2 × 2.5 mm in a flat biofilm structure	50,000 mgCOD/L [Capuno, 2007]	0.1% AOB, 0.01% NOB [Capuno, 2007]
Link 2	Diffusion of NH_4^+-N, NO_2^--N, NO_3^--N, N_2 and O_2	-	-	-	-
Inner layer	Growth and decay of ANAMMOX bacteria	10^{-9} L (Close to 0)	0.05 m^2 × 3.5 mm in a flat biofilm structure	50,000 mgCOD/L [Capuno, 2007]	10% ANAMMOX bacteria

Table 2. Kinetic parameters of bacteria for nitrogen removal

Symbol	Unit	Description	Value	Reference
μ_{AOB}	1/d	Maximum specific growth rate of X_{AOB}	2.05	Hao et al., 2002
Y_{AOB}	mgCOD/N	Growth yield of X_{AOB}	0.15	Wiesmann, 1994
$K_{NH4,AOB}$	mgNH_4^+-N/m^3	Half saturation constant of NH_4^+-N for X_{AOB}	2.4	Terada et al., 2006
$K_{O,AOB}$	mgCOD/m^3	Half saturation constant of O_2 for X_{AOB}	0.6	Terada et al., 2006
b_{AOB}	1/d	Decay rate of X_{AOB}	0.13	Koch et al., 2000
μ_{NOB}	1/d	Maximum specific growth rate of X_{AOB}	1.45	Hao et al., 2002
Y_{NOB}	mgCOD/N	Growth yield of X_{AOB}	0.041	Wiesmann, 1994
$K_{NO2,NOB}$	mgNO_2^--N/m^3	Half saturation constant of NO_2^--N for X_{AOB}	2.2	Wiesmann, 1994
$K_{O,NOB}$	mgCOD/m^3	Half saturation constant of O_2 for X_{AOB}	5.5	Koch et al., 2000
b_{NOB}	1/d	Decay rate of X_{AOB}	0.06	Koch et al., 2000
$\mu_{ANAMMOX}$	1/d	Maximum specific growth rate of $X_{ANAMMOX}$	0.08	Koch et al., 2000
$Y_{ANAMMOX}$	mgCOD/N	Growth yield of $X_{ANAMMOX}$	0.159	Strous et al., 1998
$K_{NH4,ANAMMOX}$	mgNH_4^+-N/m^3	Half saturation constant of NH_4^+-N for $X_{ANAMMOX}$	0.07	Terada et al., 2006
$K_{NO2,ANAMMOX}$	mgNO_2^--N/m^3	Half saturation constant of NO_2^--N for $X_{ANAMMOX}$	0.05	Hao et al., 2002
$K_{O,ANAMMOX}$	mgO_2/m^3	Inhibition constant of O_2 for $X_{ANAMMOX}$	0.01	Terada et al., 2006
$b_{ANAMMOX}$	1/d	Decay rate of $X_{ANAMMOX}$	0.003	Hao et al., 2002

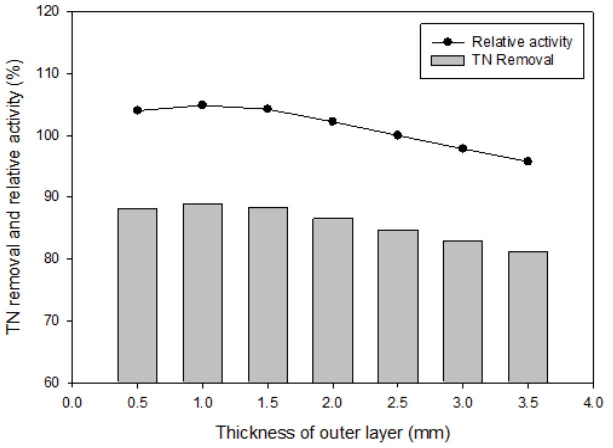

Fig. 2. The effects of the outer layer thickness on TN removal efficiency and relative activity.

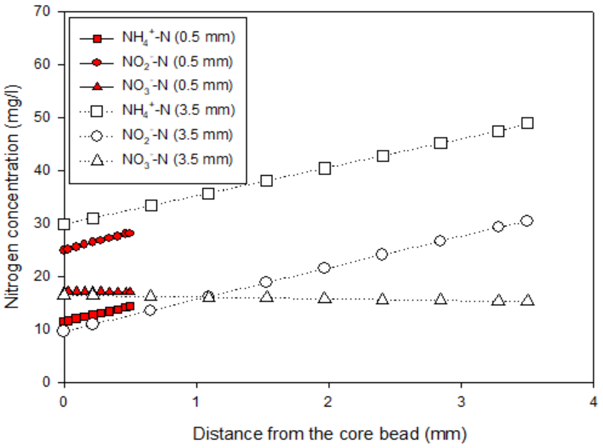

Fig. 3. The polarization of the nitrogen concentrations through the outer layer.

of AOB, NOB and ANAMMOX bacteria. This study simulates the steady-state ASSNR process treating nitrogenous wastewater including 250~750 mg-N/L of ammonium without biologically available organic carbon. The simulated bioreactor is composed of single tank with aerator with a hydraulic retention time of 1 day.

3. Results and Discussion

3.1 Effects of thickness of the outer layer

The model simulation with the AQUASIM software for the outer layer with a thickness of 2.5 mm resulted in a 84.7% of TN removal efficiency at a 0.5 mg/L of DO concentration (Fig. 2). A 0.5 mm of the thickness exhibited a high TN removal efficiency of 88.1%. The increase of the thickness to 3.5 mm resulted in a lower TN removal of 81.1%, i.e., only 4.3% reduction in the TN removal performance. Therefore, the thick layer, which provides the high mechanical strength, would be preferred to ensure the stability of ammonia-oxidation rather than a thin layer.

The difference of TN removal efficiencies was caused by the polarization of the substrate concentrations through the outer layer (Fig. 3). For the calculation of the relative activity, TN removal efficiency was divided by that of the 2.5 mm thickness. For example, for the 0.5 mm in thickness, NH_4^+-N and NO_2^--N in the bulk phase were expected to be 14.4 and 28.1 mg/L, respectively. At the interface of the core bead and the outer layer, the concentrations were decreased to 11.5 and 24.9 mg/L, respectively. The concentration polarization of a thickness of 3.5 mm results in the larger differences of 19.0 and 20.8 mg-N/L for NH_4^+ and NO_2^-, respectively. The ANAMMOX bacteria receiving lowered substrate concentrations (2.9 and 3.2 mg-N/L for NH_4^+ and NO_2^-, respectively) may exhibit a lower TN removal rate according to the Monod-type activity for the limited substrate. However, because the half saturation values for NH_4^+ and NO_2^- are extremely low as 0.07 and 0.05 mg/L in the model, the reduction in the substrate concentrations within the order of 10^1 may result in insignificant differences of the ANAMMOX activity (Hao et al., 2002; Terada et al., 2006). Generally, the rate-limiting step of wastewater using the biolfilm carriers is the diffusion of substrate into the biomass.

3.2 Effects of DO concentration

The effects of the DO concentrations in the bulk phase were investigated using the AQUASIM model. At the steady state, the optimal concentration of DO was 0.5 mg/L disregarding to the NH_4^+-N concentration (Fig. 4). The main reason for the lowered TN removal efficiency at a high DO concentration more than 0.5 mg/L was the excessive NO_2^- production (Fig. 5). Following the result of Fig. 5, which shows the steady production of NO_3^- even at a low DO concentration of 0.5 mg/L, it was expected that a high DO concentration increase the NOB activity producing NO_3^-.

3.3 Effects of biomass balance of AOB, NOB and ANAMMOX bacteria

The responses of the biofilm model according to the densities of AOB and NOB in the outer layer were examined using the AQUASIM software (Fig. 6). The low concentrations of AOB and NOB ranging from 0.1 to 10% of the biofilm were balanced for the ANAMMOX activity with a 10% biofilm density showing stable TN removal efficiencies from 82 to 84%. However, high concentrations of AOB and NOB resulted in the steep decrease in the TN removal rate due to the production of excessive NO_2^- and NO_3^-.

The balance of the densities between AOB and ANAMMOX

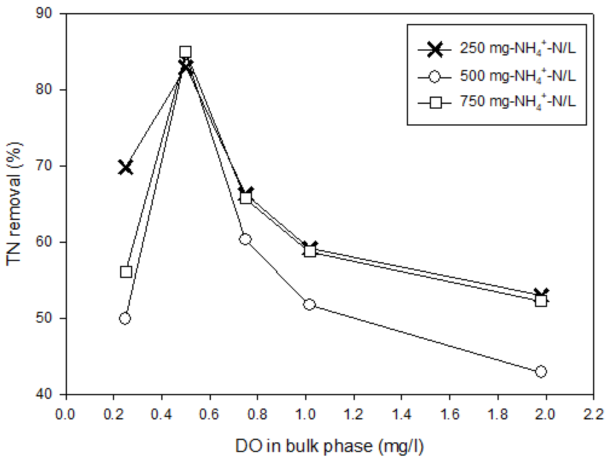

Fig. 4. Total nitrogen removal efficiency of ASSNR according to DO concentrations in the bulk phase.

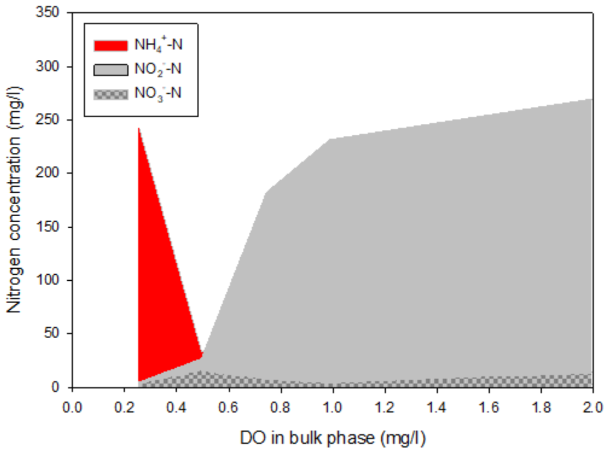

Fig. 5. Nitrogen compositions in the effluent according to the DO concentrations in the bulk phase for NH_4^+-N of 500 mg/L in the influent assuming steady state.

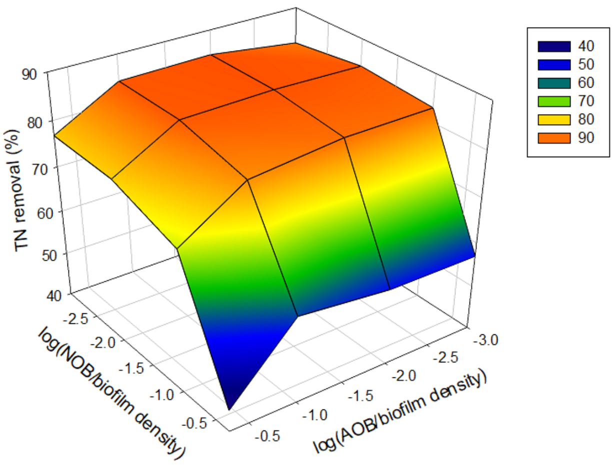

Fig. 6. Effects of the AOB and NOB concentrations on the total nitrogen removal rate in a 3-D plot.

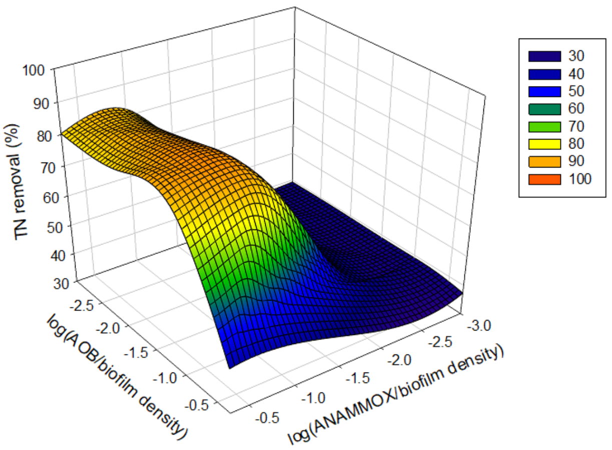

Fig. 7. Effects of the ANAMMOX bacteria and AOB concentrations on the total nitrogen removal rate in a 3-D plot.

bacteria is also the important factor to control the TN removal efficiency (Fig. 7). The preliminary factor for the optimal TN removal efficiency was the high concentration of ANAMMOX bacteria, i.e., more than 10%. At a 10% of ANAMMOX bacterial density, the optimal AOB concentration was between 1 to 10 % of biofilm density. For AOB, low concentrations less than 1% result in the significant residual NH_4^+-N while high concentrations more than 10% cause excessive NO_2^--N in the effluent. In previous study, the ecological characteristics such as high concentration of ANAMMOX bacteria has proven to be the important factor for the start-up (Tao et al., 2013). However, the suggested appropriate seeding ratio of AOB and NOB should be tested with the real biomass in batch and continuous modes.

4. Conclusion

The biofilm model simulation using an AQUASIM software was conducted for ASSAR to suggest the optimal operating conditions. Thickness has insignificant effects on the total nitrogen removal rate and the thick outer layer is preferred to ensure the high mechanical strength. The optimal DO concentration in the bulk phase is 0.5 mg/L and higher DO concentrations result in the excessive NO_2^- in the effluent. The low densities of AOB and NOB ranging from 0.1 to 10% of the biofilm were balanced for the ANAMMOX activity with a 10% biofilm density showing stable TN removal efficiencies from 82 to 84%. The preliminary factor for the optimal TN removal efficiency was the high concentration of ANAMMOX bacteria, i.e., more than 10% in the model. At a 10% of ANAMMOX bacterial density, the optimal AOB density is between 1 to 10% of biofilm density.

Acknowledgement

This work was supported by a 2-Year Research Grant of Pusan National University.

References

Bae, H., Choi, M., Chung, Y. C., Lee, S., and Yoo, Y. J. (2017). Core-shell structured poly (vinyl alcohol)/sodium alginate bead for single-stage autotrophic nitrogen removal, *Chemical Engineering Journal*, 322, 408-416.

Bae, H., Choi, M., Lee, C., Chung, Y. C., Yoo, Y. J., and Lee, S. (2015). Enrichment of ANAMMOX bacteria from conventional activated sludge entrapped in poly (vinyl alcohol)/sodium alginate gel, *Chemical Engineering Journal*, 281, 531-540.

Capuno, R. E. (2007). *Mathematical modeling for nitrogen removal via a nitritation: Anaerobic ammonium oxidation-coupled biofilm in hollow fiber membrane bioreactor and a rotating biological contactor*, Master Thesis, Virginia Polytechnic Institute and State University.

Hao, X., Heijnen, J. J., and van Loosdrecht, M. C. M. (2002). Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process, *Biotechnology and Bioengineering*, 77, 266-277.

Kim, J. H., Guo, X., and Park, H. S. (2008). Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation, *Process Biochemistry*, 43, 154-160.

Koch, G., Egli, K., Van der Meer, J. R., and Siegrist, H. (2000). Mathematical modeling of autotrophic denitrification in a nitrifying biofilm of a rotating biological contactor, *Water Science and Technology*, 41, 191-198.

Matsumoto, S., Terada, A., and Tsuneda, S. (2007). Modeling of membrane-aerated biofilm: Effects of C/N ratio, biofilm thickness and surface loading of oxygen on feasibility of simultaneous nitrification and denitrification, *Biochemical Engineering Journal*, 37(1), 98-107.

Minh, N. T., Choi, M., Park, N., and Bae, H. (2021). Critical design factors for polyvinyl alcohol hydrogel entrapping ammonia-oxidizing bacteria: Biomass loading, distribution of dissolved oxygen, and bacterial liability, *Environmental Engineering Research*, 26(2), 200127.

Piculell, M., Welander, P., Jonsson, K., and Welander, T. (2016). Evaluating the effect of biofilm thickness on nitrification in moving bed biofilm reactors, *Environmental Technology*, 37(6), 732-743.

Strous, M., Heijnen, J. J., Kuenen, J. G., and Jetten, M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, *Applied Microbiology and Biotechnology*, 50, 589-596.

Tao, Y., Gao, D. W., Wang, H. Y., de Kreuk, M., and Ren, N. Q. (2013). Ecological characteristics of seeding sludge triggering a prompt start-up of anammox, *Bioresource Technology*, 133, 475-481.

Terada, A., Lackner, S., Tsuneda, S., and Smets, B. F. (2006). Redox-stratification controlled biofilm (ReSCoBi) for completely autotrophic nitrogen removal: The effect of co- versus counter-diffusion on reactor performance, *Biotechnology and Bioengineering*, 97, 40-51.

Wanner, O., Cunningham, A. B., and Lundman, R. (1995). Modeling biofilm accumulation and mass transport in a porous medium under high substrate loading, *Biotechnology and Bioengineering*, 47, 703-712.

Wanner, O. and Morgenroth, E. (2004). Biofilm modeling with AQUASIM, *Water Science and Technology*, 49, 137-144.

Wiesmann, U. (1994). Biological nitrogen removal from wastewater, *Advances in Biochemical Engineering Biotechnology*, 51, 113-154.