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Graphical Abstract

**Modelling of Simultaneous Nitrogen and Thiocyanate Removal through Coupling
Thiocyanate-Based Denitrification with Anaerobic Ammonium Oxidation**

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ABSTRACT

Thiocyanate (SCN^-)-based autotrophic denitrification (AD) has recently been demonstrated as a promising technology that could be integrated with anaerobic ammonium oxidation (Anammox) to achieve simultaneous removal of nitrogen and SCN^- . However, there is still a lack of a complete SCN^- -based AD model, and the potential microbial competition/synergy between AD bacteria and Anammox bacteria under different operating conditions remains unknown, which significantly hinders the possible application of the coupling SCN^- -based AD with Anammox. To this end, a complete SCN^- -based AD model was firstly developed and reliably calibrated/validated using experimental datasets. The obtained SCN^- -based AD model was then integrated with the well-established Anammox model and satisfactorily verified with experimental data from a system coupling AD with Anammox. The integrated model was lastly applied to investigate the impacts of influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio and SCN^- concentration on the steady-state microbial composition as well as the removal of nitrogen and SCN^- . The results showed that the $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio in the presence of a certain SCN^- level should be controlled at a proper value so that the maximum synergy between AD bacteria and Anammox bacteria could be achieved while their competition for NO_2^- would be minimized. For the simultaneous maximum removal (>95%) of nitrogen and SCN^- , there existed a negative relationship between the influent SCN^- concentration and the optimal $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio needed.

Capsule: High-level (>95%) simultaneous removal of nitrogen and thiocyanate could be achieved through combining thiocyanate-based autotrophic denitrification and Anammox.

Keywords: thiocyanate-based autotrophic denitrification; anaerobic ammonium oxidation; microbial competition; synergy; modelling

1. INTRODUCTION

Thiocyanate (SCN^-) is a moderately toxic and chemically stable contaminant commonly found in the wastewaters of coking, plastic manufacturing, and gold mining industries (Gould et al., 2012; Kim et al., 2008; Pan et al., 2019; Sorokin et al., 2001). The direct discharge of wastewaters polluted with SCN^- would therefore represent a significant threat to aquatic species (Bhunia et al., 2000) as well as human beings (Erdoğan, 2003). Compared to chemical destruction approaches, biological degradation of SCN^- stands distinct advantages in terms of cost-effectiveness, removal efficiency, and production of secondary pollution (Felföldi et al., 2010; Gould et al., 2012). Both aerobic and anoxic technologies for biological SCN^- degradation ultimately produce ammonium (NH_4^+), sulfate (SO_4^{2-}), and carbon dioxide (CO_2) (Broman et al., 2017; Kim et al., 2011; Pan et al., 2018a; Pan et al., 2018b; Staib and Lant, 2007). Nevertheless, anoxic autotrophic degradation of SCN^- with oxidized nitrogenous species (such as nitrate (NO_3^-) in Eq. 1 or/and nitrite (NO_2^-) in Eq. 2) as the electron acceptor, which has been demonstrated experimentally (Pan et al., 2018a; Pan et al., 2018b), is superior to aerobic autotrophic degradation of SCN^- (Eq. 3), because it not only achieves denitrification but also requires no external energy input in the form of aeration. More importantly, the capability of the functional autotrophic denitrifying bacteria to respire on both NO_3^- and NO_2^- and the accompanying production of NH_4^+ create the possibility of coupling SCN^- -based autotrophic denitrification (AD) with anaerobic ammonium oxidation (Anammox). Anammox converts NO_2^- and NH_4^+ to nitrogen gas with lesser concomitant production of NO_3^- under autotrophic conditions and has been regarded as one of the most efficient and economical approaches for removing nitrogen from wastewater.

A suitable mixture of NH_4^+ , NO_2^- , and SCN^- is key for simultaneous removal of various contaminants when coupling SCN^- -based AD with Anammox (Pan et al., 2019). In such a coupling system as presented in Figure 1, AD bacteria would compete with Anammox bacteria for NO_2^- , while AD bacteria and Anammox bacteria could also work synergistically to enhance nitrogen removal through cross-feeding interactions. Therefore, the ratio between NH_4^+ and NO_2^- (i.e., $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$) for coupling SCN^- -based AD with Anammox in the presence of SCN^- , which could normally be regulated by the prepositioned partial nitrification process, should be significantly different from that for Anammox alone. Although Pan et al. (2019) studied the short-term division of labor between AD bacteria and Anammox bacteria under different initial conditions of NO_2^- , NH_4^+ , and SCN^- in batch mode, the long-term impacts of feeding NO_2^- , NH_4^+ , and SCN^- conditions on the microbial competition/synergy in the system coupling SCN^- -based AD with Anammox are yet to be investigated. The existence of intermediate elemental sulfur (S) in the conversion of SCN^- to SO_4^{2-} and the sequential denitrification process receiving electrons from SCN^- oxidation (see Figure 1), as observed experimentally by Pan et al. (2018b), further complicate the application of coupling SCN^- -based AD with Anammox. A reliable feasibility assessment of coupling SCN^- -based AD with Anammox should take into account these relevant processes as well as associated key intermediates comprehensively. In this regard, mathematical modelling serves as a useful tool. Although the Anammox model is readily available, a complete SCN^- -based AD model which covers the relevant pathways shown in Figure 1 has not been documented.

To this end, a complete SCN^- -based AD model was firstly developed, calibrated, and validated in this work, which was then integrated with the well-established Anammox model and verified using literature reported experimental datasets. The integrated model was used to

evaluate the feasibility of coupling SCN^- -based AD with Anammox in a biofilm reactor, focusing on the impacts of feeding NH_4^+ -N/ NO_2^- -N ratio and SCN^- concentration on the microbial composition of the biofilm as well as the removal of nitrogen and SCN^- . This work is expected to benefit the potential practical design and application of coupling SCN^- -based AD with Anammox in the treatment of wastewater that contains SCN^- and NH_4^+ .

2. MATERIALS AND METHODS

2.1. Experimental Datasets for Model Evaluations

As listed in Table 1, eight series of literature reported experimental datasets were used in this work for model calibration and validation.

Datasets 1-1 to 1-3 (Pan et al., 2018a) containing the time-series profiles of NO_2^- , SCN^- , NH_4^+ , and SO_4^{2-} were obtained from batch tests carried out at the initial total nitrogen (TN, i.e., sum of NO_2^- and SCN^-) concentration of 100, 200, and 300 g-N/m³, respectively, with a fixed NO_2^- -N/ SCN^- -N ratio of 3.0. The mixed liquor suspended solid (MLSS) concentration was 1000 g/m³, with the identified functional AD species (i.e., *Thiobacillus*) accounting for 38% of the denitrifying biomass. The dissolved oxygen (DO) concentration was controlled below 0.3 g/m³, and the temperature was kept at 25 °C.

Datasets 2-1 to 2-4 (Pan et al., 2018b) containing the time-series profiles of NO_3^- , NO_2^- , SCN^- , NH_4^+ , and SO_4^{2-} were obtained from batch tests performed at the initial NO_3^- -N/ SCN^- -N ratio of 1.0, 1.5, 2.0, and 3.0, respectively, with a fixed TN (i.e., sum of NO_3^- and SCN^-) concentration of 100 g-N/m³. The MLSS concentration was 500 g/m³, with the identified functional AD species (i.e., *Thiobacillus*) taking up 64.8% of the denitrifying biomass. Oxygen was removed by purging nitrogen gas, and the temperature was kept at 35 °C.

Dataset 3 (Pan et al., 2019) containing the time-series profiles of NO_3^- , NO_2^- , SCN^- , and NH_4^+ was obtained from a batch test carried out at the initial NO_2^- concentration of 75 g-N/m³ and SCN^- concentration of 25 g-N/m³. On top of the functional AD species (i.e., *Thiobacillus*) which occupied 38% of the denitrifying biomass at the MLSS concentration of 350 g/m³, Anammox bacteria were added at the MLSS concentration of 1400 g/m³ with the functional species (i.e., *Candidatus kuenenia*) identified at a relative abundance of 14.4%. The DO concentration was controlled below 0.3 g/m³, and the temperature was kept at 30 °C.

2.2. Development of Thiocyanate-Based AD Model

The SCN^- -based AD model was constructed based on the pathways as presented in Figure 1. Specifically, sulfur in SCN^- is firstly oxidized to elemental sulfur and further to SO_4^{2-} , consistent with Xu et al. (2015), Xu et al. (2016), and Yang et al. (2016) who reported elemental sulfur as the intermediate in the biological oxidation of S^{2-} to SO_4^{2-} . The possible roles of SCN^- and sulfite are not included in the model as they were not detected in the reported SCN^- -based AD systems (Pan et al., 2018a; Pan et al., 2018b). Production of NH_4^+ is the accompanying process associated with oxidation of SCN^- to elemental sulfur. Electrons generated from oxidation of SCN^- and elemental sulfur drive denitrification which is assumed to mainly take place in two steps: reduction of NO_3^- to NO_2^- (denitratation) and further reduction of NO_2^- to nitrogen gas (denitrification). Therefore, as tabulated in Table S1 in the supporting information (also contains Tables S2 and S3 and Figure S1), in addition to the inevitable biomass decay, four oxidation-reduction reactions are considered for the SCN^- -based AD model, all of which lead to biomass growth. As sulfur in SCN^- is the real electron donor, SCN^- is expressed in terms of S rather than N in the model, while biomass is recorded in the form of chemical oxygen demand (COD). Yield coefficient, Y_{AD} , is used to build the

relationship between substrate consumption and product/biomass formation. Correct mass balance of all components was ensured through continuity checking according to Hauduc et al. (2010). Double Monod-type kinetic equation is used to describe the enzymatic kinetic processes.

2.3. Calibration and Validation of Thiocyanate-Based AD Model

In consideration of the fair number of parameters associated with the SCN⁻-based AD model as listed in Table S2, the well-established parameter values should be used as much as possible, which would also reduce the possibility of over-parametrization. Therefore, using the heuristic approach (i.e., process knowledge) as applied by Lu et al. (2018), four sensitive parameters were identified, i.e., $\mu_{\text{SCN},\text{NO}_3}^{\text{AD}}$ (maximum specific growth rate of AD bacteria on SCN⁻ and NO₃⁻), $\mu_{\text{SCN},\text{NO}_2}^{\text{AD}}$ (maximum specific growth rate of AD bacteria on SCN⁻ and NO₂⁻), $\mu_{\text{S},\text{NO}_3}^{\text{AD}}$ (maximum specific growth rate of AD bacteria on S and NO₃⁻), and $\mu_{\text{S},\text{NO}_2}^{\text{AD}}$ (maximum specific growth rate of AD bacteria on S and NO₂⁻), which should be calibrated using experimental data. The other parameters were directly adopted from literature reported values (see Table S2).

A stepwise protocol was applied to calibrate and validate the developed model, i.e., estimating the values of the four identified sensitive parameters. The first step was to estimate $\mu_{\text{SCN},\text{NO}_2}^{\text{AD}}$ and $\mu_{\text{S},\text{NO}_2}^{\text{AD}}$ using Datasets 1-1 to 1-3 with supply of NO₂⁻ and SCN⁻ only which involved SCN⁻-based denitrification (Reaction 2 of Table S1) and S-based denitrification (Reaction 4 of Table S1). On top of the successful completion of the first step, $\mu_{\text{SCN},\text{NO}_3}^{\text{AD}}$ and $\mu_{\text{S},\text{NO}_3}^{\text{AD}}$ were estimated in the second step using Datasets 2-1 to 2-4 which further included SCN⁻-based denitrification (Reaction 1 of Table S1) and S-based denitrification (Reaction 3 of

Table S1) due to the provision of NO_3^- and SCN^- . It should be noted that the data points for the first two hours of Datasets 2-1 to 2-4 were discarded due to the possible incomplete removal of oxygen that inhibited the reduction of NO_3^- , as claimed by Pan et al. (2018b).

Being applied to both model calibration and validation, the initial AD bacteria concentration was determined based on the mixed liquid volatile suspended solid (MLVSS) concentration (assumed as 85% of the measured MLSS concentration, which is commonly used in biological wastewater treatment environments (Randall et al., 1998)) together with the relative abundance of AD bacteria (see Table 1). A conversion factor of 1.42 (i.e., ratio between COD and VSS) was applied to determine biomass concentration in terms of COD (Contreras et al., 2002). Arrhenius equation was applied to account for the role of varying temperature between datasets. With 30 °C as the reference temperature for the developed SCN^- -based AD model, the conversion of the growth rate (μ) and decay rate (b) in the temperature range between 25 °C and 35 °C was assumed to follow $\mu(T) = \mu(30) * \theta^{(T-30)}$ or $b(T) = b(30) * \theta^{(T-30)}$, where θ was inferred to be 1.1 for AD based on the work of Di Capua et al. (2016).

Parameter estimation was conducted using a modified AQUASIM 2.1d (Reichert, 1998). Uncertainty evaluation was performed according to Batstone et al. (2003) with a 95% confidence level for significance testing and parameter uncertainty analysis.

2.4. Verification of Model Integrating Thiocyanate-Based AD with Anammox

The calibrated/validated SCN^- -based AD model was then integrated with the Anammox model, with the structure and parameters (at the reference temperature of 30 °C) being included in Tables S1 and S2. Dataset 3 with simultaneous contributions of AD and

Anammox was eventually used to assess the validity of the integrated model without further adjustment in parameter values. In line with the aforementioned model calibration and validation process, the AD bacteria and Anammox bacteria concentrations were set using same approach, and temperature was also considered using Arrhenius equation (see Table S2).

2.5. Assessment of Coupling Thiocyanate-Based AD with Anammox in a Biofilm Reactor

The verified integrated model was then applied to simulate the implementation of coupling SCN^- -based AD with Anammox in a biofilm reactor, which is particularly suitable for co-culturing slow-growing autotrophs and has been widely applied to study microbial competition/synergy in the long-term operation (Di Capua et al., 2015). As summarized in Table S3, the simulated biofilm reactor had a total volume of 1 m^3 , consisting of 0.9 m^3 of bulk liquid and 0.1 m^3 of biofilm. Assuming a uniform steady-state biofilm thickness of $100 \mu\text{m}$ without consideration of its variation with locations, the total area of biofilm surface was 1000 m^2 . The inflow rate into the reactor was fixed at $0.083 \text{ m}^3/\text{h}$, giving rise to a hydraulic retention time of 12 h.

Scenario 1 was designed to investigate the impact of influent $\text{NH}_4^+ \text{-N}/\text{NO}_2^- \text{-N}$ ratio on the coupling system. Therefore, the influent SCN^- and the sum of NH_4^+ and NO_2^- were controlled at 25 g-N/m^3 and 150 g-N/m^3 , respectively. The respective concentrations of NH_4^+ and NO_2^- were adjusted systematically, rendering an influent $\text{NH}_4^+ \text{-N}/\text{NO}_2^- \text{-N}$ ratio between 0:10 and 9:1. Scenario 2 was further proposed to study the influence of influent SCN^- concentration on the $\text{NH}_4^+ \text{-N}/\text{NO}_2^- \text{-N}$ ratio leading to the optimal treatment performance of the biofilm reactor. To this end, with a varying $\text{NH}_4^+ \text{-N}/\text{NO}_2^- \text{-N}$ ratio at the sum of NH_4^+ and NO_2^- of 150 g-N/m^3 , the influent SCN^- concentration was adjusted between 5 and 40 g-N/m^3 . For both simulation

scenarios, steady-state nitrogen removal and SCN^- removal were both used as criteria to measure the treatment performance of the coupling system. To avoid duplicate representation, nitrogen content in SCN^- was not accounted for when calculating the removal efficiency of TN, which was termed as sum of NO_3^- -N, NO_2^- -N, and NH_4^+ -N hereafter.

Both simulation scenarios assumed an initial biofilm thickness of 1 μm and were run long enough to reach the steady state which was controlled by the surface detachment velocity equation reported by Lackner et al. (2008). The resistance approach based on Fick's first law was used to describe the transport of dissolved components into or out of the biofilm. Detailed information about the reactor setup and biofilm characteristics is provided in Table S3.

3. RESULTS

3.1. Calibration and Validation of Thiocyanate-Based AD Model

Figures 2A and 2B illustrate the first-step calibration results of the denitrification processes of the developed SCN^- -based AD model using Datasets 1-1 and 1-2. At the initial NO_2^- -N/ SCN^- -N ratio of 3.0, although SCN^- was completely removed, NO_2^- still remained at the end of the batch tests. With the simultaneous consumption of SCN^- and NO_2^- , SO_4^{2-} and NH_4^+ were formed. The great agreement between model predictions and experimental measurements in Figures 2A ($R^2 = 0.98$) and 2B ($R^2 = 0.99$) supported the goodness of the calibration for the denitrification processes. The validation of the denitrification processes was performed using Dataset 1-3, with results presented in Figure 2C. The good match between the model predicted and measured trends ($R^2 = 0.99$) verified the validity of $\mu_{\text{SCN},\text{NO}_2}^{\text{AD}}$ and $\mu_{\text{S},\text{NO}_2}^{\text{AD}}$ for the developed SCN^- -based AD model, which were estimated at $0.0045 \pm 0.0001 \text{ g}/(\text{g} \cdot \text{h})$ and $0.157 \pm 0.021 \text{ g}/(\text{g} \cdot \text{h})$, respectively.

The second-step calibration results of the denitrification processes of the developed SCN^- -based AD model using Datasets 2-1 and 2-2 are exhibited in Figures 3A and 3B. As explained by Pan et al. (2018b), the data points for the first two hours (the shadow regions) were excluded in this work due to the possible interference of oxygen remaining in the beginning of the batch tests. At the relatively low initial NO_3^- -N/ SCN^- -N ratios of 1.0 (Dataset 2-1) and 1.5 (Dataset 2-2), both NO_3^- and SCN^- were nearly completely removed at the end of the batch tests. In addition to SO_4^{2-} and NH_4^+ , NO_2^- was also formed. Nevertheless, after NO_3^- was completely removed in Dataset 2-1 (Figure 3A), NO_2^- started to be consumed, confirming the contribution of the denitrification processes. The fine match between model predicted and experimentally measured profiles in Figures 3A ($R^2 = 0.88$) and 3B ($R^2 = 0.92$) proved the goodness of calibrating the denitrification processes. Figures 3C and 3D show the validation results of the denitrification processes using Datasets 2-3 and 2-4. Because of the elevated NO_3^- -N/ SCN^- -N ratios (i.e., 2.0 and 3.0), SCN^- was completely consumed while some NO_3^- remained at the end of the batch tests. The satisfying agreement between model predictions and experimental measurements in Figures 3C ($R^2 = 0.96$) and 3D ($R^2 = 0.92$) supported the validity of calibrated values of $\mu_{\text{SCN},\text{NO}_3}^{\text{AD}}$ (0.0048 ± 0.0003 g/(g·h)) and $\mu_{\text{S},\text{NO}_3}^{\text{AD}}$ (0.0064 ± 0.0002 g/(g·h)) for the denitrification processes of the developed SCN^- -based AD model.

The 95% confidence regions for $\mu_{\text{SCN},\text{NO}_2}^{\text{AD}}$ and $\mu_{\text{S},\text{NO}_2}^{\text{AD}}$ and for $\mu_{\text{SCN},\text{NO}_3}^{\text{AD}}$ and $\mu_{\text{S},\text{NO}_3}^{\text{AD}}$ in combination with their uncorrelated confidence intervals obtained are delineated in Figures S1A and S1B, respectively. The good reliability and identifiability of the four estimated parameter values were supported by the small uncorrelated confidence intervals of parameters lying inside the correlated confidence region.

3.2. Verification of Model Integrating Thiocyanate-Based AD with Anammox

Figure 4 illustrates the verification results of the calibrated/validated SCN^- -based AD model integrated with the Anammox model using Dataset 3. With the consumption of NO_2^- and SCN^- , neither NO_3^- nor NH_4^+ was observed in significant amount throughout the batch test, proving the simultaneous contributions of AD and Anammox. The fact that the integrated model could capture the experimentally measured profiles to a high level ($R^2 = 0.94$) manifested the validity and capability of the integrated model to describe the joint presence of AD and Anammox metabolisms. The evaluated integrated model could therefore be reliably applied in the following section to assess the feasibility of coupling SCN^- -based AD with Anammox in a biofilm reactor.

3.3. Effect of NH_4^+ -N/ NO_2^- -N Ratio on Coupling Thiocyanate-Based AD with Anammox

The long-term impact of influent NH_4^+ -N/ NO_2^- -N ratio on the microbial composition of the biofilm as well as TN and SCN^- removal efficiencies (Scenario 1) is delineated in Figure 5. At the SCN^- supply of 25 g-N/m^3 in Scenario 1, the maximum NO_2^- consumption and NH_4^+ production via SCN^- -based AD were fixed. An influent NH_4^+ -N/ NO_2^- -N ratio of 0:10 (i.e., no external supply of NH_4^+) meant a complete utilization of SCN^- -based AD but an oversupply of NO_2^- as the electron acceptor for Anammox bacteria. Consequently, AD bacteria dominated the biofilm at a relative abundance of 76% with the coexistence of Anammox bacteria (24%). Nearly complete removal of SCN^- (98.3%) was achieved, while the TN removal efficiency was only 52.8%. With the influent NH_4^+ -N/ NO_2^- -N ratio increasing to 2:8, the contribution of Anammox was enhanced through the elevated external NH_4^+ supply. Therefore, the relative abundance of Anammox bacteria increased while that of AD bacteria decreased, leading to the increasing TN removal without hindering the SCN^- removal. At the

influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio of around 3:7, a balanced synergy between AD bacteria (57%) and Anammox bacteria (43%) was obtained, generating a TN removal efficiency of 99.0% and a concomitant SCN^- removal efficiency of 95.0%. Further increase in the influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio until 9:1 intensified the competition between AD bacteria and Anammox bacteria for NO_2^- availability so that Anammox bacteria gained advantage over AD bacteria. Consequently, the relative abundance of AD bacteria decreased to 5% while that of Anammox bacteria increased to 95% at the influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio of 9:1. As only NO_2^- was completely used, both the TN and SCN^- removal decreased with the increasing influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio.

3.4. Effect of SCN^- on Optimal $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ Ratio for Coupling Thiocyanate-Based AD with Anammox

Figure 6 illustrates the long-term impact of influent SCN^- concentration on the optimal $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio leading to highest TN removal as well as corresponding microbial composition of the biofilm and accompanying SCN^- removal efficiency (Scenario 2). Simultaneous high-level removal of TN ($99.0\pm0.3\%$) and SCN^- ($95.6\pm1.2\%$) could be obtained at the influent SCN^- concentration between 5 and 40 g-N/m^3 . However, the corresponding optimal $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio decreased with the increasing influent SCN^- concentration. This was because of the increasing need of AD bacteria for NO_2^- as the electron acceptor at the increasing influent SCN^- concentration. Therefore, in terms of the microbial community, the relative abundance of AD bacteria increased while that of Anammox bacteria decreased.

4. DISCUSSION

Different from the model describing aerobic autotrophic biodegradation of SCN^- that has been studied previously (Du Plessis et al., 2001; Hung and Pavlostathis, 1999), the SCN^- -based AD model studied in this work creates the opportunity to assess the treatment of SCN^- -containing wastewater using anoxic technologies, which are generally regarded as more cost-effective. Through a well-designed two-step calibration and validation process, the SCN^- -based AD model was first reliably obtained in this work, as proved in Figures 2 and 3. Compared to the work of Pan et al. (2019) where the sequential reduction of NO_3^- to nitrogen gas via NO_2^- and elemental sulfur as the intermediate were not considered, the SCN^- -based AD model developed in this work could better represent the true metabolic pathways, thus enabling more accurate model-based evaluations.

The developed SCN^- -based AD model was integrated with the Anammox model, which, after being verified with literature reported experimental data (see Figure 4), was then used to assess the microbial competition/synergy between AD bacteria and Anammox bacteria in a coupling biofilm reactor under different operating conditions. It should be pointed out that the potential inhibition of SCN^- on Anammox bacteria was not considered in the model of this work, which certainly could be modified upon the future availability of relevant information. The good match between the model predicted and measured trends presented in Figures 2 to 4 proved the applicability of the model parameters obtained in this work under the concentration conditions of SCN^- , NH_4^+ , and NO_2^- studied and the temperature conditions between 25 °C and 35 °C. As shown in Figure 5, the influent $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio in the presence of a certain SCN^- concentration should be controlled at a proper level so that the maximum synergy between AD bacteria and Anammox bacteria was achieved while their competition for NO_2^- was minimized. Only under this operating condition would the maximum removal of nitrogen and SCN^- be achieved simultaneously. However, when the

influent SCN^- level changed, the corresponding $\text{NH}_4^+ \text{-N/NO}_2^- \text{-N}$ ratio for simultaneous maximum removal (>95%) of TN and SCN^- should change as well. In general, there existed a negative relationship between the influent SCN^- concentration and the optimal $\text{NH}_4^+ \text{-N/NO}_2^- \text{-N}$ ratio needed (Figure 6). Therefore, in real application, the extent of partial nitrification of wastewater containing SCN^- and NH_4^+ should be properly regulated so that a good $\text{NH}_4^+ \text{-N/NO}_2^- \text{-N}$ ratio in the presence of SCN^- is provided for the coupling system. Moreover, compared with single Anammox process, the integration of SCN^- -based AD with Anammox enhances nitrogen removal through utilizing NO_3^- produced by Anammox and adds on SCN^- removal, although it requires a lower $\text{NH}_4^+ \text{-N/NO}_2^- \text{-N}$ ratio and hence a higher extent of the preceding partial nitrification. In brief, the integrated process still stands a promising alternative for treating industrial wastewaters containing substantial amount of SCN^- and NH_4^+ .

The applicability of the integrated model developed in this work could be further extended to predicting the fate of other background pollutants such as COD or biochemical oxygen demand, if present, through integration with well-established process models such as activated sludge models. Future work could also focus on the extension of the integrated model with relevant process kinetics to describe the role of pH and the formation of complex from the reactions between SCN^- and metal ions, as the availability of SCN^- , NH_4^+ , or/and NO_2^- which directly affects the treatment performance is heavily dependent on both pH dynamics and the potential presence of metal ions in wastewater.

5. CONCLUSIONS

Using literature reported experimental data, a complete SCN^- -based AD model taking into account not only relevant processes but also associated key intermediates was firstly developed/calibrated/validated and then integrated/verified with the well-established

Anammox model. The assessment results by using the integrated model showed that the $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio in the presence of a certain SCN^- level should be controlled at a proper value so that the maximum synergy between AD bacteria and Anammox bacteria was achieved whilst their competition for NO_2^- was minimized. For the simultaneous maximum removal (>95%) of nitrogen and SCN^- , there existed a negative relationship between the influent SCN^- concentration and the optimal $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ ratio needed. The findings obtained would benefit the potential practical design and application of coupling SCN^- -based AD with Anammox in the treatment of wastewater that contains SCN^- and NH_4^+ .

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DECLARATIONS OF INTEREST

None.

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Table and Figure Legends

Table 1. Experimental data for model calibration, validation, and evaluation

Figure 1. Pathways of SCN^- -based autotrophic denitrification (AD) and Anammox and their interactions.

Figure 2. (A and B) Calibration and (C) validation results of denitrification processes of the SCN^- -based autotrophic denitrification (AD) model.

Figure 3. (A and B) Calibration and (C and D) validation results of denitrification processes of the SCN^- -based autotrophic denitrification (AD) model.

Figure 4. Verification results of the model integrating SCN^- -based autotrophic denitrification (AD) with Anammox.

Figure 5. Long-term impact of NH_4^+ -N/ NO_2^- -N ratio on microbial composition of the biofilm as well as TN and SCN^- removal efficiencies (Scenario 1).

Figure 6. Long-term impact of SCN^- level on optimal NH_4^+ -N/ NO_2^- -N ratio leading to highest TN removal as well as corresponding microbial composition of the biofilm and accompanying SCN^- removal efficiency (Scenario 2).

Table 1. Experimental data for model calibration, validation, and evaluation

Dataset	Initial SCN ⁻ (g-N/m ³)	Initial NO ₂ ⁻ (g-N/m ³)	Initial NO ₃ ⁻ (g-N/m ³)	MLSS-AD (g/m ³)	Abundance of AD	MLSS-AN (g/m ³)	Abundance of AN	Temperature (°C)	Source
1-1	25	75	0	1000	38%	0	0	25	Pan et al. (2018a)
1-2	50	150							
1-3	75	225							
2-1	50	0	50	500	64.8%	0	0	35	Pan et al. (2018b)
2-2	40		60						
2-3	33.3		66.7						
2-4	25		75						
3	25	75	0	350	38%	1400	14.4%	30	Pan et al. (2019)

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Highlights

- A complete thiocyanate-based autotrophic denitrification (AD) model was developed.
- Thiocyanate-based AD model was evaluated with integration of Anammox model.
- Relationship between thiocyanate and $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ for maximum removal was studied.

The authors declare no conflict of interest.

ACCEPTED MANUSCRIPT