Impact of Coexistence of Sludge Flocs on Nitrous Oxide Production in a Granule-based Nitrification System: A Model-based Evaluation

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ABSTRACT

A common operational status of granule-based reactor is the inevitable coexistence of sludge flocs. Such hybrid system could have a profound impact on nitrous oxide (N_2O) production in nitrifying process. In this work, a mathematical model is employed to evaluate the key role of the coexistence of sludge flocs on N_2O production in a granule-based nitrifying system for the first time, by considering both nitrifier denitrification and hydroxylamine oxidation pathways. The modelling results show that the N_2O production gradually decreases with the increase of the percentage of sludge flocs in the total biomass (10 – 60%). More N_2O is tended to be generated in sludge flocs which has lower N_2O production capacity compared to granular biomass, thus lowering the total N_2O production. The relative contributions of two N_2O production pathways are only affected by bulk dissolved oxygen (DO) for the sludge flocs in the hybrid system, whereas those are affected by both bulk DO and the fractions of sludge flocs for the granular biomass. The results reveal a substantial effect of the coexistence of sludge flocs on N_2O production in granule-based nitrifying process, which should not be ignored in future design and operation.

KEYWORDS: nitrifying system; N_2O production pathways; N_2O emission; granular sludge; sludge flocs; mathematical modeling

1. INTRODUCTION

Nitrous oxide (N_2O) is a highly potent fugitive greenhouse gas (GHG) that contributes significantly to climate change. Over a 100-year horizon, 1 ton of N_2O will cause a warming effect equivalent to ca. 300 tons of carbon dioxide (CO_2) (Stocker et al., 2013). Also, it is a dominant ozone layer depleting substance (Ravishankara et al., 2009). The global average atmospheric N_2O concentration increased from approximately 270 ppb in 1750 to 319 ppb in 2005 (Law et al., 2012). It is estimated that more than 50% of N_2O emission is due to anthropogenic activities (Stocker et al., 2013). Wastewater treatment plants (WWTPs) can be one of significant anthropogenic sources of N_2O. There is plentiful nitrogen flow into WWTPs, creating great potential for N_2O production. Even though a minor portion of nitrogen compounds is converted to N_2O during biological nitrogen removal (BNR) process, it would lead to significant GHG emission (Vasilaki et al., 2019).

BNR involves nitrification and denitrification. N_2O production can arise from incomplete denitrification by heterotrophic denitrifiers under certain conditions. However, there is increasing evidence that ammonia oxidizing bacteria (AOB) is the principal contributor to N_2O emission from WWTPs (Desloover et al., 2011, Kampschreur et al., 2007, Kampschreur et al., 2008, Okabe et al., 2011, Yu et al., 2010). During autotrophic nitrification, there are two major mechanisms involved in N_2O generation by AOB, namely the hydroxylamine (NH_2OH) pathway and nitrifier denitrification pathway (Law et al., 2013, Peng et al., 2014, Wunderlin et al., 2012). The first includes incomplete oxidation of NH_2OH to NO with N_2O as a by-product, and the second involves the sequential reduction of nitrite (NO_2^-) to N_2O as an end product with nitric oxide (NO) as an intermediate. Different responses of these two pathways to different operational conditions have been reported (Chen et al., 2018, Su et al., 2017). For example, dissolved oxygen (DO) has been identified as a very important factor to trigger the shift of the two N_2O pathways during nitrification (Ni et al., 2013b, Peng et al., 2014). The nitrifier denitrification pathway is promoted by limiting DO concentrations (Tallec et al., 2006), while the hydroxylamine pathway is favoured at elevated DO concentrations (Chen et al., 2019).

Aerobic granular sludge process has been widely used for BNR due to its high nitrification rate, excellent settling velocity and effective biomass retention in the reactor (Rathnayake et al., 2013, Zhu and Wilderer, 2003), which offers considerable advantage over the suspended floccular activated sludge. As such, it has been successfully implemented into full-scale applications, with improved effluent quality, better sludge volume index (SVI) of 45 – 48 mL g^{-1}, as well as 30 – 33% less reactor volume and 58 – 63% lower energy requirement than the existing conventional activated sludge plant (Li et al., 2014, Pronk et al., 2015, Świątczak et al., 2018). In comparison to loose and suspended flocs in the activated sludge process, granules are compact and dense aggregates with a fast settleability that are particularly suitable to enrich the slow-growth AOB during BNR (Chen et al., 2019, Lemaire et al., 2008). It has been found that different levels of sludge flocs can still be present in granule-based processes, suggesting the coexistence of these two types of biomass aggregates in granule-based reactors (Hubaux et al., 2015, Vlaeminck et al., 2010, Winkler et al., 2012). Due to its less diffusion limitation than granular biomass, higher volumetric nitrification rates can even be obtained at low DO in sludge flocs (Veuillet et al., 2014). Therefore, small amounts of sludge flocs in a granule-based BNR system could largely affect the nitrogen removal efficiency (Leix et al., 2016). As such, it is reasonable to hypothesize that such a mixture of floc and granule aggregates may also affect the N_2O production pathway and thereby the emission capacity. However, the impact of the coexistence of flocs on granule-based BNR systems in terms of N_2O production has not been yet analysed experimentally due to the system complexity and uncertainty, i.e., the accurate separation of flocs and granules for N_2O analysis.

Mathematical modelling has been proven to be a powerful tool towards understanding metabolic mechanism of N_2O production by AOB during BNR (Ni et al., 2014, Sabba et al., 2015). N_2O models incorporating both nitrifier denitrification pathway and hydroxylamine pathway have been developed to describe the mechanisms and contributions of N_2O production pathways in the sludge flocs-based rectors (Peng et al., 2014, Pocquet et al., 2016). Also, similar models have been utilized to unveil N_2O production pathways in the pure granule-based systems (Chen et al., 2019, Lu et al., 2018). However, little effort has yet been dedicated to revealing the N_2O production mechanisms in a hybrid nitrification system with both granular biomass and sludge flocs being presented simultaneously.

Therefore, this study aims to conduct a systematic model-based evaluation on the key role of coexistence of sludge flocs on N_2O production in a granule-based nitrification system, through implementing a two-pathway model for N_2O production by AOB in such a hybrid system. Extensive simulations were carried out to investigate the underlying mechanisms of N_2O production and pathway differentiation due to the impacts of percentage fraction of sludge flocs and DO levels in a granule-based nitrification system coexisting with sludge flocs. The results of this work could potentially provide a better understanding of the N_2O production in granule-based nitrifying reactor and hence improving the operation of such system.

2. MATERIALS AND METHODS

2.1 Two-pathway N_2O model

The N_2O model employed in this work describes relevant microbial processes of AOB and nitrite oxidizing bacteria (NOB) in nitrification processes (Figure 1). Based on the previous studies (Chen et al., 2019, Pocquet et al., 2016), a two-pathway model by considering both nitrifier denitrification and hydroxylamine oxidation pathways was applied to describe AOB-associated N_2O production in five enzymatic reactions (Figure 1): (1) ammonium (NH_4^+) oxidation to NH_2OH by ammonia monooxygenase (AMO), (2) NH_2OH oxidation to nitric oxide (NO) by hydroxylamine oxidoreductase (HAO), (3) NO oxidation to NO_2^- by HAO, (4) NO reduction to N_2O by nitric oxide reductase (Nor) coupled with NH_2OH oxidation to NO_2^-, and (5) NO_2^- reduction to N_2O by nitrite reductase (Nir) and Nor accompanied with NH_2OH oxidation (Figure 1). Reactions 2 and 4 describe the NO and N_2O production via the hydroxylamine pathway. Reaction 5 describes N_2O production via the nitrifier denitrification pathway.

Overall, this model synthesizes relevant reactions in conversion of NH_4^+, NH_2OH, NO_2^-, NO_3^-, NO, N_2O and oxygen (O_2) and describes the relationships among three biomass groups: AOB, NOB and residual inert biomass. Abiotic N_2O production pathway is not considered in the model as it only contributes less than 3% to overall N_2O emissions in typical nitritation systems (Su et al., 2019a). Other biological processes, such as aerobic NO_2^- oxidation to nitrate (NO_3^-) by NOB (Figure 1) and endogenous respiration by AOB and NOB, are incorporated in the model with standard Activated Sludge Model (ASM) kinetic expressions. In order to reduce the model complexity, ionized NH_4^+ and NO_2^- are assumed to be the substrate of AOB and NOB, instead of unionized free ammonia (NH_3) and free nitrous acid (HNO_2), respectively (Chen et al., 2019). The inhibitions of NH_3 and HNO_2 on microbial growth are not considered as their concentrations are relatively low in comparison to inhibitory levels (Pocquet et al., 2016). The inhibition effect of DO on the nitrifier denitrification pathway is added in Reaction 5 according to Chen et al. (2019) and Ni et al. (2011). The detailed model kinetics and stoichiometry are presented in Tables S1 and S2 (Supporting information, SI).

2.2 Modelling the hybrid nitrifying reactor with granules and flocs

A 1-D multispecies diffusion-reaction model was used to describe the coexistence of flocs and granules in the hybrid nitrifying system through connecting a biofilm compartment to a completely mixed reactor compartment in AQUASIM (Reichert, 1994). The former compartment acts as a hybrid biological reactor and the latter compartment serves as a sedimentation tank. The effluent with the biomass detached from the granule surface of the biofilm compartment settles in sedimentation tank, from which the flocs are recirculated back to the biofilm compartment by implementing a bifurcation function. Such set-up enables the simultaneous growth of both granular and floccular biomass in the hybrid nitrifying reactor (Hubaux et al., 2015, Liu et al., 2018). Through controlling the recirculating ratios, different pre-determined percentages of the coexisting sludge flocs in the total biomass (i.e., sludge flocs and granular biomass) in the hybrid reactor can be achieved.

Specifically, flocs in the system are homogeneously distributed in the reactor without mass transfer limitation. In contrast, granules in the system are considered to be mass diffusion limited, and are modeled according to Ni et al. (2009) and Liu et al. (2015). The granules are assumed to be spherical biofilms and uniform in size, with the number and size of granules fixed. There is no biomass migration in the granules. Only radial diffusion transport is accounted for in the biofilm, which is described by Fick's law. The diffusion coefficient is constant and mass transfer boundary layer is not considered. For the soluble components involved in the biological reactions, the first step is their diffusion into the granule where the reactions take place. Discretization in time of the partial-differential equation was used to describe the reaction-diffusion kinetics in such a spherical granule (Peng et al., 2017). Biomass in the granules is detached from the particulate phase to the bulk phase where they are homogeneously distributed and remain active before being removed proportionally to the effluent flow (Hubaux et al., 2015). The detachment rate was set as $u_F \cdot \left(\frac{L_f}{L_{Fmax}}\right)^2$ (u_F: growth velocity of biofilm, L_f: the biofilm thickness and L_{Fmax}: maximum biofilm thickness, 500 μm) (Liu et al., 2018).

2.3 Simulation approaches and scenarios

The stoichiometric and kinetic parameters used for this model are adapted from literature and listed in Table S3, with same values being applied for both sludge flocs and granular biomass. The mass transfer coefficients for NH_4^+, NH_2OH, NO_2^-, NO_3^-, NO, N_2O and O_2 in the granules are modified from Sabba et al. (2015) with the consideration of a reduction factor of 0.8 (Table S3). Biomass density in the granules was set to 50000 g/m^3. The active biomass in the granules, i.e., AOB and NOB, had an equal spilt-up fraction at the beginning of the simulation, as the initial species distributions would not affect the steady-state results (Laspidou and Rittmann, 2004, Rittmann and Manem, 1992). The granules had a porosity of 80% and the granule size grew from an initial 20 μm to 500 μm. The influent ammonium concentration was 50 mg-N/L. The reactor volume was 30 L and the flow rate was 5 L/h, resulting in a hydraulic retention time (HRT) of 6 h. Model simulations were then performed to reach system steady state through varying the DO concentrations in the aqueous phase (0.2, 0.35, 0.5, 0.75, 1, 2 and 3 mg/L) and percentages of sludge flocs in the total biomass (i.e., sludge/sludge plus granules, 0%, 10%, 20%, 30%, 40%, 50% and 60%). The grid number was set to 20.

3. RESULTS

3.1 N_2O production in a putative granule-based nitrifying system without sludge flocs at different bulk DO level

The model was firstly applied to describe the N_2O production and production pathways in a putative granular sludge nitrifying system without sludge flocs at different bulk DO level (Figure 2), in order to serve as a benchmark value and compare with later simulation cases with the coexistence of sludge flocs in the system. The predicted N_2O production factor from such system decreases from 3.5% to 1.6% with the increase of DO from 0.2 to 3 mg/L (Figure 2a), along with an ammonium oxidation efficiency between 93% – 98%. The initial increase of bulk DO from 0.2 to 0.5 mg/L leads to a linear decrease of N_2O production factor. Later increase of bulk DO from 0.75 to 3 mg/L results in a gradual decrease in N_2O production factor, which almost reaches plateaus between 2 – 3 mg/L (Figure 2a). Therefore, bulk DO levels significantly affect N_2O production (Pijuan et al., 2014) in putative granular nitrifying system, with higher N_2O production occurring at lower DO. The reason could be attributed to higher NH_2OH accumulated within granular biomass at such lower DO conditions, which leads to more N_2O production (Sabba et al., 2015). However, this is inconsistent with Peng et al. (2017) who observed increase of bulk DO from 0.25 to 3 mg/L largely promoted N_2O production by AOB in a simulated granular partial nitrification system. This is likely because they considered the inhibition function of nitrite on both N_2O production pathways by AOB and simulated significant increase of nitrite accumulation from 100 – 250 mg-N/L with the increase of DO. In comparison, nitrite accumulation is lower than 2 mg-N/L in all simulations of this work.

For N_2O production pathways, the nitrifier denitrification pathway (60%) dominates over the NH_2OH oxidation pathway (40%) at a DO level of 0.2 mg/L. At a DO level of ca. 0.25 – 0.3 mg/L, the contributions of these two pathways are roughly equal. Similar results have been observed by Ali et al. (2016) that the contributions of both N_2O production pathways were nearly identical at low DO levels in a nitritation granular reactor. Further increase of bulk DO levels from 0.35 to 3 mg/L motivates the NH_2OH oxidation pathway (i.e., 62% to 95%) and diminishes the nitrifier denitrification pathway (i.e., 38% to 5%). This is in agreement with Peng et al. (2014) that higher bulk DO levels promote the NH_2OH oxidation pathway but suppress the nitrifier denitrification pathway.

3.2 Impact of coexistence of sludge flocs on total N_2O production from a granule-based nitrification system

The model was then applied to simulate the percentage of sludge flocs in the total biomass (10 – 60%) on N_2O production from a granule-based system with varying bulk DO levels (Figure 3), which reveals that even small amounts of sludge flocs (i.e., 10%) in the system could largely affect the N_2O dynamics compared to the case without sludge flocs above (Figure 2).

Similar to the putative granular sludge nitrifying system (Figure 2), higher DO levels lowers total N_2O production in such a hybrid system with the coexistence of both flocs and granules (Figure 3). Similar results have also been observed in a previously reported putative nitrifying granular sludge systems that N_2O production factor decreased from 10.6% to 2.4% with an increase of DO from 0.2 – 3 mg/L (Peng et al., 2014).

Meantime, it is clearly revealed that the N_2O production gradually decreases with the increase of the percentages of sludge flocs in the total biomass (Figures 3a – 3f). It should be note that the N_2O production factor in a putative granular sludge nitrifying system decreases from 3.5% to 1.6% with the increase of DO from 0.2 to 3 mg/L (Figure 2a). In contrast, the production factors decrease from 1.2 – 0.7% at a sludge percentage of 10% (Figure 3a) to 0.5 – 0.25% at a sludge percentage of 60% (Figure 3f) under DO from 0.2 to 3 mg/L, along with an ammonium oxidation efficiency between 95% – 98%. It has been accepted that nitrifying granules or biofilms could lead to more N_2O production than suspended floccular sludge systems under the same condition (Chen et al., 2019, Sabba et al., 2015). The main cause is the accumulation and transport of the intermediate NH_2OH from the outer aerobic to inner anoxic zones in the biofilm/granule system (Sabba et al., 2015). In the inner anoxic zones, the electrons generated from NH_2OH oxidation by AOB is solely used for nitrite reduction to N_2O, considering there is no competition with oxygen due to the limited transfer of oxygen within the granule (Peng et al., 2017). In comparison, the uniformly distributed NH_2OH across the sludge system lowers such N_2O production potential. Therefore, the granular systems with coexistence of suspended sludge show much lower N_2O production than putative granule-based system.

Sensitivity analyses were conducted to evaluate the model structure and to investigate the most determinant biokinetic parameters relating to N_2O production in the system using the AQUASIM built-in algorithms, with results shown in Figures S1 in SI. The N_2O production in both sludge flocs and granules is most sensitive to $q_{AOB,N2O,NN}$ (maximum N_2O production rate by NH_2OH oxidation pathway), $q_{AOB,HAO}$ (maximum rate for HAO reaction), $\mu_{AOB,HAO}$ (maximum AOB growth rate) and $K_{NO,AOB,HAO}$ (AOB affinity constant for NO from HAO). In the future application of the model, it is not practical to measure all of the numerous biokinetic parameters involved. Therefore, accurate determination of those particularly sensitive to the performance in combination with reported values of other parameters could significantly reduce the calibration efforts while generating reliable results.

3.3 Impact of coexistence of sludge flocs on the contributions of sludge flocs and granular biomass to total N_2O production

As shown in Figure 4a, the N_2O production from sludge in the granule-based system increases with increasing percentages of sludge flocs from 10% to 60% as expected, with that from granules showing an opposite trend (Figure 4b). For example, 10% of sludge flocs contribute to 15 – 18% of total N_2O production, and 60% of sludge flocs contribute to 64 – 83% of total N_2O production in the system. Therefore, more N_2O is tended to be produced in sludge flocs compared to granules (Figure 4). The main reason could be the biomass segregation that AOB tend moves from the granule to sludge (Laureni et al., 2019). Sludge, as a floccular material, is thus directly exposed to bulk DO, while granules, as a type of biofilm, are oxygen limited. This provides a selection pressure for segregation of microbial populations (Liu and Tay, 2002, Sabba et al., 2018, Todt et al., 2016). Also, the community structure variation between sludge and granules has been reported in a hybrid system, i.e., faster growing microorganisms (e.g., AOB) tend to live in suspended sludge, and slower growing microorganisms (e.g., NOB) are predominant in the granules (Desloover et al., 2011). As such, the contribution of sludge to total N_2O production increases due to the increase of N_2O-producing AOB in the sludge.

In addition, bulk DO also plays an important role in regulating the contributions of N_2O production between sludge flocs and granules. The contribution of sludge flocs to total N_2O production firstly increase and then gradually declines with the increase of DO from 0.2 to 3 mg/L (Figure 4a). This is because initial increase of bulk DO leads to the proliferation of AOB in the sludge. But further increase causes fully penetration of DO in the granule and thereby AOB stop relocation from the granules to sludge. Notably, the contribution of granules to N_2O production decreases with the increasing amount of flocs (Figure 4b) and therefore the total N_2O production of the granule-based system decreases significantly (Figure 3), as nitrifying biofilms could produce more N_2O than suspended sludge under similar conditions (Chen et al., 2019). As such, although the contribution from sludge flocs increased, the total N_2O production in the system decreased interestingly.

3.4 Impact of coexistence of sludge flocs on N_2O production pathway dynamics within the granular biomass

Previous studies indicated that both nitrifier denitrification and NH_2OH oxidation pathways could be important for N_2O production, which would dynamically change depending on conditions such as DO (Ishii et al., 2014, Ni et al., 2013b, Tallec et al., 2006). Therefore, the model was used to evaluate the contributions of two N_2O production pathways within the granules of a granule-based nitrification system due to the coexistence of sludge flocs under different bulk DO concentrations (Figure 5). With the increase of DO from 0.2 to 3 mg/L, the contribution of the nitrifier denitrification pathway in the granule decreases from ca. 53 – 80% to 5% (Figure 5a), while the contribution of the NH_2OH oxidation pathway in the granule increases from ca. 20 – 47% to 95% (Figure 5b). It has been reported that the penetration limitation of DO could result in a predominant role of the nitrifier denitrification pathway in the granule (Sabba et al., 2015). Therefore, the nitrifier denitrification pathway dominates over the NH_2OH oxidation pathway under DO of ca. 0.2 – 0.35 mg/L. The contributions of these two pathways become equal under DO of ca. 0.35 – 0.5 mg/L. Further increase of DO to 3 mg/L leads to the domination of the NH_2OH oxidation pathway. Similar results have been reported by Rathnayake et al. (2013), that the nitrifier denitrificaiton pathway in a granular sludge reactor contributed 35% of N_2O production at an initial bulk DO of 2 mg/L, and it latter decreased to be equal to the NH_2OH oxidation pathway with the decrease of DO.

Also, at a bulk DO between 0.2 and 0.5 mg/L, higher fraction of coexisting sludge flocs leads to a higher contribution of the nitrifier denitrification pathway in the granule (Figure 5a, and Figure 2b where flocs are 0%). The main reason is that the increasing amount sludge flocs (i.e., 60%) consumes more DO in the bulk phase and this causes intensified DO penetration limitation in the granule (Hubaux et al., 2015). Such effect was insignificant with further increase of DO from 0.75 to 3 mg/L. Higher bulk DO results in better penetration of oxygen within the granule and thereby makes granules into "floc-like" materials (Chen et al., 2018). This explains the negligible impact of varying sludge percentages on the contributions of pathways at higher DO. Therefore, both the nitrifier denitrification and NH_2OH oxidation pathways contribute to N_2O production in the granule of a hybrid system, and the relative contributions of these two pathways are affected by bulk DO and fractions of sludge flocs.

3.5 Impact of coexistence of sludge flocs on N_2O production pathway dynamics within the sludge flocs

The contributions of N_2O production pathways in the sludge flocs of a granule-based nitrification system were also evaluated under various fractions of sludge flocs and bulk DO concentrations. In contrast to what occurs in granules, there is no impact of fractions of sludge flocs on N_2O production pathway dynamics in the sludge. As the bulk DO level increases from 0.2 to 3 mg/L, the contribution of nitrifier denitrification decreases from ca. 37% to 5%, accompanied with an increase in the contribution of the NH_2OH oxidation pathway from 63% to 95%. Such trend has been found in many studies of sludge systems, that DO has a positive effect on the NH_2OH oxidation pathway which therefore increases with DO (Chen et al., 2018, Peng et al., 2014, Pocquet et al., 2016). However, the NH_2OH oxidation pathway dominates in the sludge flocs under all the conditions simulated in this study. This is inconsistent with Tallec et al. (2006) and Chen et al. (2018) who found that the nitrifier denitrification was the major N_2O production contributor at DO ranges of 0.1 – 3 mg/L. The differences can be attributed to the presence of granules in our system, which promotes the movement of N_2O-producing AOB from the granules to sludge. The NH_2OH oxidation pathway is thus enhanced in the flocs due to higher DO availability in the bulk liquid phase.

4. DISCUSSION

The granular process has been widely used in WWTPs and the coexistence of sludge flocs in granular reactor is often observed. This model-based study, for the first time, identified that such effect could lead to unexpected profiles in terms of N_2O production and pathways. The results clearly reveal the significant impact of coexistence of sludge flocs on N_2O production from a granule-based nitrification system, where the N_2O production gradually decreases with the increase of the percentages of sludge flocs in the total biomass while the nitrification efficiency is not largely impacted. The N_2O production factor from a putative granular sludge nitrifying system in this study decreases from 3.5% to 1.6% between 0.2 and 3 mg/L DO, which is comparable to some N_2O emission data from both full-scale and modeled attached-growth nitrifying systems. For example, Pijuan et al. (2014) observed increasing the DO from 1 to 4.5 mg/L resulted in a decrease on N_2O emissions from 6 to 2.2% in a granular nitritation reactor treating ammonium-rich wastewater, while Sabba et al. (2015) reproduced such data via a modeling study. Bollon et al. (2016) estimated average N_2O emission factor of 2.26% ± 0.46% from full-scale nitrifying biofilters. In contrast, our further simulation indicated that the coexistence of 10 – 60% sludge flocs decreased N_2O production to 1.2 – 0.25%. Notably, 1% decrease in N_2O production could lead to about 30% decrease in carbon footprint during wastewater treatment processes (Law et al., 2012). Therefore, the information of this work might be helpful to effectively mitigate N_2O production in such hybrid systems.

In a hybrid system with both flocs and granules, N_2O is tended to be generated in sludge (Figure 4) which has lower N_2O production capacity compared to granules. The key difference between granule- and sludge-based processes are intermediate diffusion patterns due to the inherent properties of granules and sludge (Chen et al., 2019). NH_2OH produced by AOB can diffuse according to substrate gradients along the biofilm to act as a sink for NH_2OH, but uniformly distribute in the sludge (Sabba et al., 2015). Such distribution results in significantly higher N_2O production in the inner anoxic granule but much lower N_2O production in the sludge under similar conditions (Peng et al., 2017). Therefore, increasing amounts of coexisting sludge will alleviate the N_2O production potential in the hybrid system.

Meanwhile, increasing the availability of DO will reduce the anoxic zone where the electrons generated from NH_2OH oxidation is solely used for nitrite reduction to N_2O (Chen et al., 2018), which also leads to lower contributions of the nitrifier denitrification pathway but higher contributions of the NH_2OH oxidation pathway to N_2O production in both granules and sludge (Figures 5 and 6). This is in agreement with many previous studies that the contribution of the nitrifier denitrification pathway increases as DO decreases whereas the contribution of NH_2OH oxidation pathway increases with the DO (Kampschreur et al., 2007, Peng et al., 2014). In addition, the nitrifier denitrification pathway in the granule inversely correlates with the N_2O production factor. Increasing DO or sludge fraction could result in a higher contribution of the nitrifier denitrification pathway in the granule and thereby alleviate N_2O production.

It is known that heterotrophic bacteria are able to produce and consume N_2O, especially for treating real wastewater as indicated by Bollon et al. (2016) and Blomberg et al. (2018). However, our preliminary simulation results have indicated heterotrophic bacteria contribute negligible N_2O production/consumption in an anoxic granule-based autotrophic nitrifying system (granules: 90%, sludge flocs: 10%, DO: 0.2 mg/L). As such, heterotrophic denitrification is not specifically considered in our model in order to reduce the model complexity. Such assumptions are justifiable since heterotrophic bacteria growing cell lysate is very limited due the lack of organics in the influent and AOB/NOB are slow growing microorganisms, which have been confirmed in previous studies (Peng et al., 2014, Peng et al., 2015). Due to this, the liquid-gas transfer of N_2O is not considered as there is no N_2O sink in the model. Therefore, the N_2O produced can stay in the liquid phase to be discharged with the effluent and be considered as N_2O emitted. However, the role of heterotrophic denitrifiers could be largely enhanced if there are organic matter in the influent (mimicking a nitrifying system treating real wastewater), particularly under low DO and abundant granules conditions, such impacts could be readily included into our model by incorporating the well-established stoichiometric and kinetic heterotrophic denitrification parameters (Hiatt and Grady, 2008) in future research.

Also, the pH would affect both abiotic and biotic N_2O production pathways in nitritation systems. Su et al. (2019a) pointed out that abiotic N_2O production only contributes <3% to overall N_2O emission in typical nitritation systems. On the other hand, pH would affect biotic N_2O production rates (Blum et al., 2018), but such impact is not significant at pH between 7.5 and 8.5, typical pH values for nitrification systems (Domingo-Felez and Smets, 2020, Law et al., 2011, Su et al., 2019b). Therefore, the effect of pH on N_2O production is not considered in this model, especially as the goal of this study is to firstly identify the impact of coexistence of sludge flocs on N_2O production in such a granule-based nitrification system. The impacts of pH could be considered following Domingo-Felez and Smets (2020) if necessary in future studies.

In addition, the impact of biomass density in the granule on N_2O production is not simulated in this study. It would be generally expected that the increase of densities inside a biofilm of constant thickness would increase the active biomass, and thereby N_2O production. However, our previous model has already shown that increasing biofilm density has negligible influence on the N_2O production in similar biofilm systems (Ni et al., 2013a, Peng et al., 2016).

The objective of this work is to provide insights into the key role of the coexistence of sludge flocs on N_2O production in a granule-based nitrifying system. Ideally, the above goal in this study would be achieved if the model could be calibrated using experimental data. This is unfortunately not possible at present due to the lack of data. We have therefore chosen to conduct a simulation study by integrating well-established models describing key biological processes to assess the contributions of N_2O production in both granules and sludge. We recognize that without being validated with data, the model predictions are preliminary and remain to be verified. However, we believe the preliminary results will already support our understanding in this system. Further efforts should be devoted to conducting experimental work to support the hypotheses produced by this modeling work in future.

The implications of this study would help to better understand and develop the effective strategies to reduce the accumulation of N_2O caused by the coexistence of sludge flocs in hybrid granule-based nitrifying systems at WWTPs. It is suggested that increasing the solid retention time (SRT) to enlarge the amount of floccular sludge could effectively lower the N_2O production while maintaining the satisfactory nitrogen removal efficiency for a granule-based nitrifying system. Similar performance can also be readily achieved by shortening HRT. This will accordingly increase hydrodynamic shear forces required for the formation of granules for wastewater treatment (Ren et al., 2009). Increasing shear forces will stimulate bacteria to produce more extracellular polymer substances and thus facilitate the formation of compact granules with higher nitrogen removal capacity (Tang et al., 2009). Meanwhile, higher shear forces would detach more biomass from granules and thus form more sludge flocs in the system, which could in turn decrease the N_2O production. Additionally, N_2O emission in such hybrid system can be manipulated by control of DO values. Higher DO could also lower the N_2O production, with an optimal point of DO existed. The performance would not change above that DO point and thus too high DO would consume more energy. Therefore, DO should be carefully at an appropriate low range to reduce N_2O emission. In fact, it would be expected that more nitrite would be normally available for nitrifier denitrification pathway for N_2O production if it is less consumed by NOB. However, our additional simulations with AOB only in the system revealed that there is no significant differences on N_2O emissions, contributions to total N_2O production by sludge flocs or granules, and production pathways, as compared to the current AOB+NOB system (data not shown). Thus, the implication of this study can also be applied to hybrid granule-based partial-nitritation systems.

5. CONCLUSION

In summary, a mathematical model is used to evaluate the impacts of the coexistence of sludge flocs on N_2O production in a granule-based nitrification system for the first time. The modelling results suggested a substantial effect of the role of sludge flocs on N_2O production in such a hybrid system. N_2O is tended to be generated in sludge flocs which has lower N_2O production capacity compared to granules, thus lowering the total N_2O production. Increasing the fraction of sludge flocs in the hybrid system only results in a higher contribution of the nitrifier denitrification pathway in the granule but not affects that in the sludge. The results of this study could be useful in practical applications to mitigate N_2O emissions from granule-based nitrification systems. Increasing SRT and HRT would enlarge the fraction of sludge flocs and thereby reduce N_2O emission. DO should be carefully controlled at an appropriate low range to further reduce N_2O emission and save energy cost.

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Figure Captions

Figure 1. Schematic of the N_2O production as well as AOB and NOB reactions involved in the model.

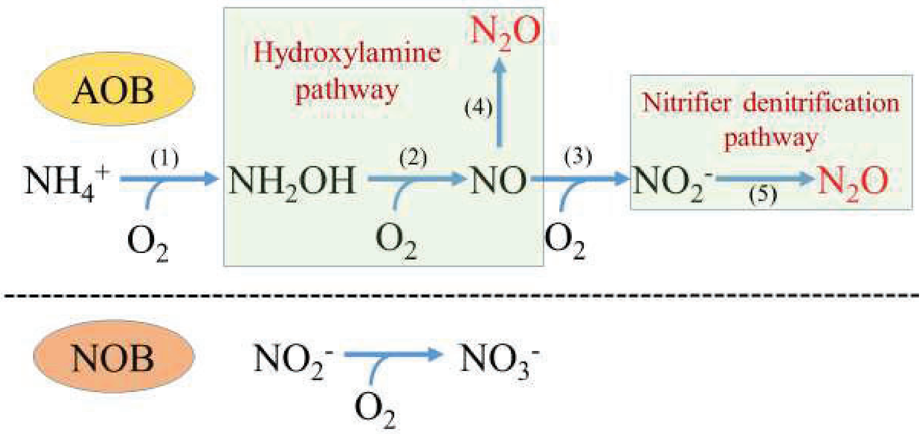
Figure 2. N_2O production and pathways from a putative granular nitrification system without sludge flocs at different bulk DO levels.

Figure 3. Total N_2O production from a granule-based nitrification system with the coexistence of (a) 10%, (b) 20%, (c) 30%, (d) 40%, (e), 50% and (f) 60% sludge flocs (bulk DO varying from 0.2 to 3 mg/L).

Figure 4. Contributions to total N_2O production from (a) sludge flocs and (b) granular biomass, in a granule-based nitrification system with the coexistence of 10 – 60% sludge flocs (bulk DO varying from 0.2 to 3 mg/L).

Figure 5. Shift of (a) nitrifier denitrification and (b) NH_2OH oxidation pathway N_2O production pathways in the granules, of a granule-based nitrification system with the coexistence of 10 – 60% sludge flocs (bulk DO varying from 0.2 to 3 mg/L).

Figure 6. Shift of (a) nitrifier denitrification and (b) NH_2OH oxidation pathway N_2O production pathways in the sludge flocs, of a granule-based nitrification system with the coexistence of 10 – 60% sludge flocs (bulk DO varying from 0.2 to 3 mg/L).



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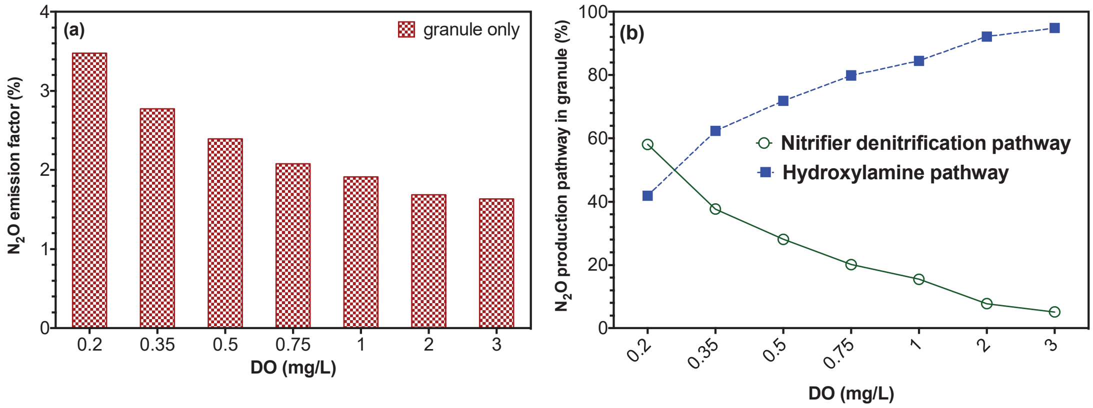

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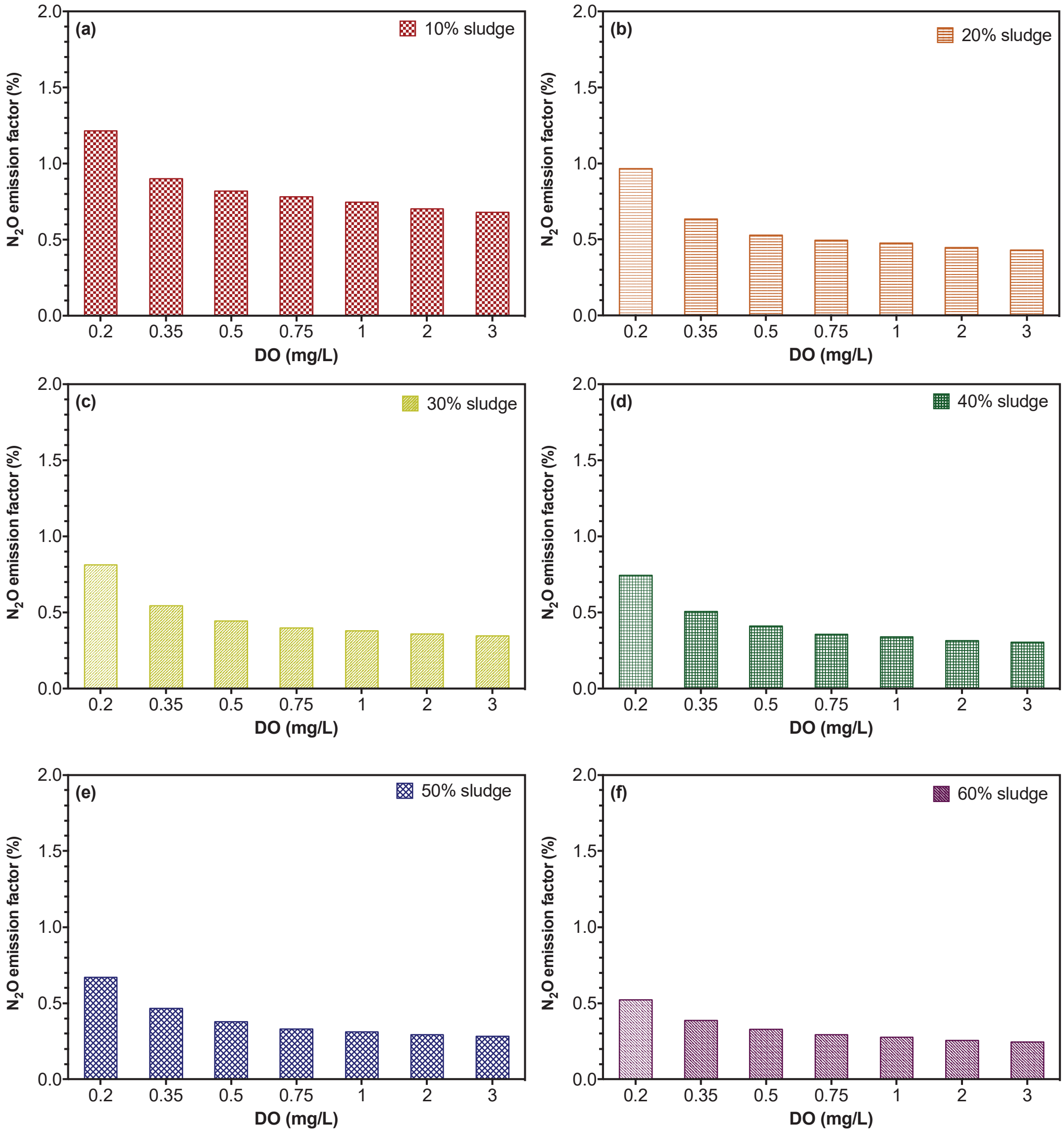

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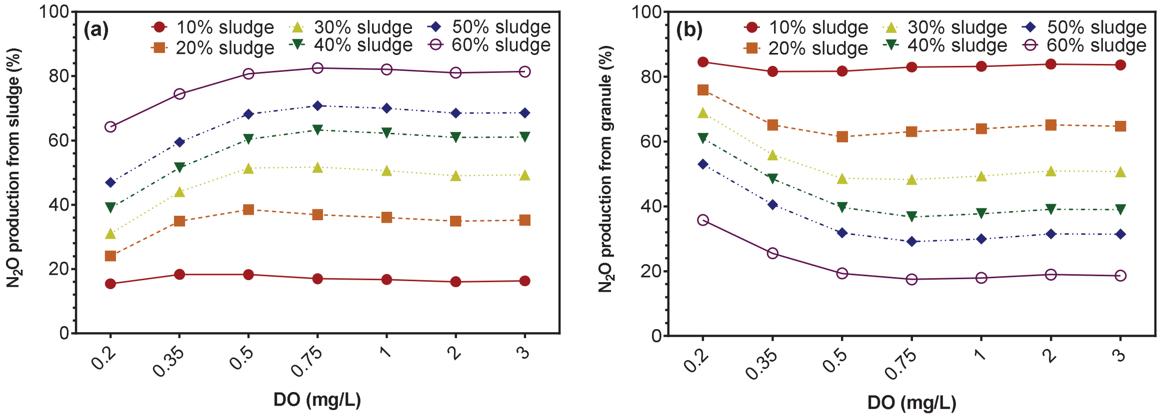

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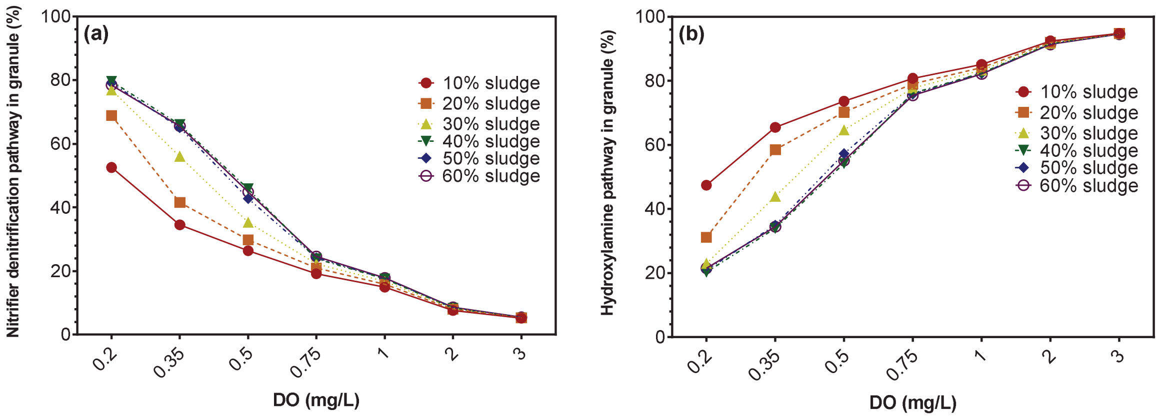

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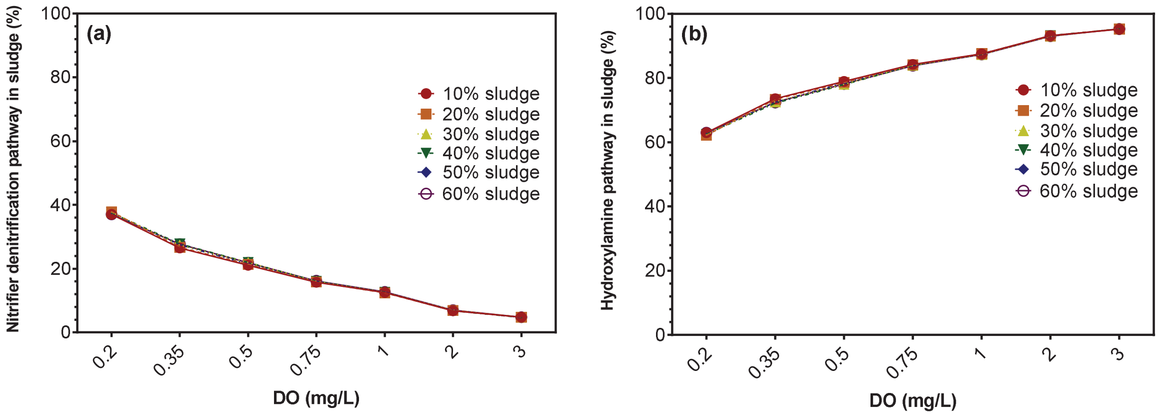

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