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**Impact of Granule Size Distribution on Nitrous Oxide Production in Autotrophic
Nitrogen Removal Granular Reactor**

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ABSTRACT

This work applied an approach with reactor compartmentation and artificial diffusion to study the impact of granule size distribution on the autotrophic granular reactor performing partial nitrification and anaerobic ammonium oxidation with focus on the nitrous oxide (N_2O) production. The results show that the microbial community and the associated N_2O production rates in the granular structure are significantly influenced by the granule size distribution. Heterotrophic bacteria growing on microbial decay products tend to be retained and contribute to N_2O consumption in relatively small granules. Ammonium-oxidizing bacteria are mainly responsible for N_2O production via two pathways in granules of different sizes. Under the conditions studied, such heterogeneity in the granular structure disappears when the number of granule size classes considered reaches more than 4, where heterotrophic bacteria are completely outcompeted in the granules. In general, larger granules account for a higher portion of the net N_2O production, while the trend regarding the volumetric contribution of each granule size class changes with a varied number of granule size classes, due to the different contributions of relevant N_2O production pathways (with the heterotrophic denitrification pathway being the most decisive). Overall, with the increasing extent of granule size distribution, the nitrogen removal efficiency decreases slightly but consistently, whereas the N_2O production factor increases until the number of granule size classes reaches 4 or above. Practical implications of this work include: i) granules should be controlled as well-distributed as possible in order to obtain high nitrogen removal whilst minimizing N_2O production; ii) granule size distribution should be considered carefully and specifically when modelling N_2O production/emission from the autotrophic nitrogen removal granular reactor.

Keywords: Autotrophic nitrogen removal granular reactor; granule size distribution; modelling; N_2O production factor

1. INTRODUCTION

The combination of partial nitrification (PN) by ammonium-oxidizing bacteria (AOB) and anaerobic ammonium oxidation (Anammox) represents a sustainable autotrophic nitrogen removal technology that has been commonly regarded as superior to the conventional nitrification/denitrification process for its cost-effectiveness in energy supply, sludge disposal, and resource recovery (Jetten et al., 2001; Kartal et al., 2010; Kuenen, 2008; Sliekers et al., 2002). Considering the relatively slow growth of Anammox bacteria and the difference between AOB and Anammox bacteria in the affinity for oxygen, granular sludge is particularly suitable for retaining AOB and Anammox bacteria simultaneously and sufficiently in a single-stage autotrophic nitrogen removal system, where the creation of spatially separated aerobic and anoxic zones is driven by dissolved oxygen (DO) concentration gradient inside granules (Lu et al., 2018).

Nevertheless, the widely-documented nitrous oxide (N_2O) emissions (Ali et al., 2016; Blum et al., 2018; Castro-Barros et al., 2015; Domingo-Félez et al., 2014; Joss et al., 2009; Kampschreur et al., 2009; Ma et al., 2017; Sliekers et al., 2002) have brought about a significant uncertainty to the sustainable application and operation of PN/Anammox systems. N_2O is known as a potent greenhouse gas with a global warming potential 265 times higher than carbon dioxide (IPCC, 2014) as well as a major scavenger of stratospheric ozone, thus contributing to the ozone layer depletion (Ravishankara et al., 2009). In PN/Anammox systems, AOB have been regarded as the main contributor to N_2O production through the hydroxylamine (NH_2OH) pathway or/and the AOB denitrification pathway, while Anammox bacteria have been identified as incapable of producing N_2O (Kartal et al., 2006). Moreover, despite the inorganic influent conditions, heterotrophic bacteria (HB) might still grow on microbial decay products accumulated in the granular structure (Lotti et al., 2014; Mozumder

et al., 2014; Ni et al., 2012). Consequently, in addition to its primary positive contribution to nitrogen removal through reducing nitrate produced by Anammox bacteria, the co-occurrence of HB might play a significant role in regulating N_2O production in autotrophic nitrogen removal systems, as N_2O has been proved as an obligate intermediate of the sequential heterotrophic denitrification process (Hanaki et al., 1992; Ni et al., 2016). Further complicating the implementation of the autotrophic nitrogen removal granular system is the uncertainty associated with granule size distribution. Granule size is an important indicator of the granule surface to volume ratio that determines the mass transport of solutes in the granular biofilm, thus regulating the reactor performance (Chen et al., 2019; Volcke et al., 2012). A previous study by Liu et al. (2017) have demonstrated the influence of granule size distribution on the nitrogen removal performance of a similar granular reactor. However, little has been known regarding the impact of granule size distribution on the N_2O production from the autotrophic granular reactor performing PN/Anammox.

Mathematical modelling, a reliable tool to study biological wastewater treatment systems (Kuriqi, 2014; Kuriqi et al., 2016), has been commonly applied to explore N_2O production from various reactor configurations. However, when it comes to modelling granular reactors, a uniform granule size is assumed in most cases, even though the actual granules tend to follow certain size distribution (Volcke et al., 2012; Zhu et al., 2018b). So far, two main approaches have been proposed to simulate granule size distribution in granular reactors using Aquasim (Reichert, 1998), simulation software widely used for one-dimensional multi-substrate and multi-species biofilm modelling. Based on proper compartmentation of a reactor that captures difference in biofilm thickness, the first approach by Volcke et al. (2012) cross-links compartments using advective links, while the second approach by Baeten et al. (2017) connects compartments with diffusive links to mimic complete mixing in bulk liquid.

The availability of these approaches makes it possible to systematically assess the N_2O production from the autotrophic granular reactor in consideration of granule size distribution.

To this end, a previously-developed N_2O model capable of describing relevant mechanisms in autotrophic nitrogen removal systems (Chen et al., 2019) was applied in this work. With granule size distribution being considered in accordance with Baeten et al. (2017), the impact of granule size distribution on N_2O production was investigated for the autotrophic nitrogen removal granular reactor. This work is expected to gain useful insights into 1) future control over granule size characteristics in order to mitigate N_2O production/emission from the autotrophic nitrogen removal granular reactor and 2) good practice in modelling N_2O production/emission from the autotrophic nitrogen removal granular reactor based on the extent of granule size distribution.

2. MATERIALS AND METHODS

2.1. Model Describing Nitrous Oxide Production during Autotrophic Nitrogen Removal

The mathematical model whose validity has been verified using experimental datasets in our previous work (Chen et al., 2019) was directly adopted in this work to describe the joint mechanisms of relevant contributing microorganisms, including AOB, nitrite-oxidizing bacteria (NOB), HB, and Anammox bacteria, in an autotrophic nitrogen removal granular reactor. In brief, three pathways are considered for N_2O production, which include 1) the AOB denitrification pathway, where N_2O is the final product, 2) the NH_2OH pathway by AOB, where N_2O is a byproduct of incomplete oxidation of NH_2OH via NO to NO_2^- , and 3) the sequential reduction of bioactive nitrogen oxides to N_2 by HB, where N_2O is an intermediate.

Collectively, as shown in Table S1 in the supporting information (SI), the model contains 15 state variables: 6 solid components and 9 soluble components. The interactive relationships among these components mediated by different contributing microorganisms involve altogether 17 process reactions (Table S2 in the SI), leading to substrate consumption, product formation, and biomass growth/decay. The corresponding process reaction rates, which are described by either substrate-dependent Monod equation or first-order equation with respect to biomass, are listed in Table S3 (SI). Together with their values, units, and sources, the stoichiometric and kinetic parameters involved in the model are summarized in Table S4 (SI).

2.2. Approach to Modelling Granule Size Distribution

To model granule size distribution in a well-mixed granular reactor, the configuration presented in Fig. 1 was implemented in Aquasim (Reichert, 1998) through compartmentation of the reactor and utilization of the diffusive link. As explained by Baeten et al. (2017), the introduced mass transfer rate (R_d) of a compound between two diffusively linked compartments, which is analogous to the well-known gas-liquid mass transfer model, is calculated by $R_d = q_{ex}(S_1 - S_2)$, where S_1 is the concentration in the first compartment, S_2 is the concentration in the second compartment, and q_{ex} is a predefined, sufficiently high exchange coefficient. Specifically, depending on the number of granule size classes ($n \geq 2$) to be considered, the implementation (i.e., Fig. 1) in Aquasim could be achieved by the following steps:

- 1) Create one biofilm compartment for every granule size class as well as one mixed compartment.
- 2) Connect the inflow (Q) and outflow (Q) to the mixed compartment, and connect every biofilm compartment to the mixed compartment with a diffusive link.

- 3) Divide the total reactor volume (V) equally among all the different compartments, each therefore having a volume of $V/(n+1)$.
- 4) Divide the total number of granules (N) equally among all the different biofilm compartments, each therefore having N/n granules.
- 5) Assign the same, sufficiently high diffusive exchange coefficient (e.g., $1000000 \text{ m}^3/\text{d}$) to every soluble and solid component for all the diffusive links, thus ensuring nearly identical (maximum allowable difference of 1%) concentrations of all components in the bulk liquid of all the different compartments.

As discussed in Baeten et al. (2017), this approach with diffusive links (i.e., Fig. 1) is advantageous over the previous advection-based approach proposed by Volcke et al. (2012), which uses reasonably high advective links to connect the bulk liquid of compartments to mimic the mixing, in its elimination of the trade-off between mixing and correct mass balance, thus leading to higher reliability and efficiency, wider applicability, easier implementability, and a lower risk for numerical errors in Aquasim. With this novel approach, the impact of granule size distribution on N_2O production in the autotrophic nitrogen removal granular reactor could be reliably investigated, which is detailed in the following section.

2.3. Reactor Setup and Modelling Scenarios

The referencing settings in our previous work (Chen et al., 2019) which could lead to a total nitrogen (TN) removal efficiency of around 90% in the autotrophic nitrogen removal granular reactor operated in the continuous mode were directly applied in this work. Specifically, as indicated in Table S5 (SI), the total reactor volume (V) was considered to be fixed at 1 m^3 , including 0.2 m^3 of granules and 0.8 m^3 of bulk liquid. The inflow and outflow rate (Q) was considered to be $1 \text{ m}^3/\text{d}$, corresponding to a hydraulic retention time of 1 day. The inflow was

modelled to only constitute NH_4^+ at the concentration of 500 g-N/m^3 in the absence of biodegradable organics. The bulk DO concentration was kept constant at 0.25 g/m^3 .

To study the impact of granule size distribution, five simulation scenarios were performed in this work (see Fig. S1 in the SI). The nearly normal distribution of granule size used to design the simulation scenarios was generated with reference to a lab-scale granular reactor and is presented in Fig. S2 in the SI. Table 1 summarizes the detailed features of reactors with different granule size classes considered for the five scenarios. Scenario 1 investigated the autotrophic granular reactor with a uniform granule size of $500 \mu\text{m}$ (radius), representing one granule size class. Through dividing the total volume of granules (i.e., 0.2 m^3) by the volume of each granule, the total number of granules (N) was calculated at 3.82×10^8 . Scenarios 2 to 5 probed into the autotrophic granular reactor with 2 to 5 granule size classes, respectively. The granule size (radius) in each granule size class was selected so that the total granule volume of all granule size classes equaled to 0.2 m^3 . This setting ensured the same average granule size for all the simulations scenarios, thus making the extent of granule size distribution the only variable to be studied. For the sake of clear presentation, granule size class is referred to as size class X/Y , where X stands for the X th granule size class while Y represents the total number of granule size classes modelled for a particular autotrophic granular reactor, in the following sections. The granule size (radius) of size class $(X+1)/Y$ was defined as significantly higher than that of size class X/Y .

For each simulation scenario, the initial concentrations of all 9 soluble components in the autotrophic granules as well as the bulk liquid were assumed to be zero, while each of the four contributing microorganisms and the abiotic organics (inert and slowly biodegradable organics) was assumed to take up 20% of the initial solid chemical oxygen demand (COD) in

the granules. All the simulation scenarios assumed a biomass density of 100000 g-COD/m³ and a granule porosity of 0.75 (Koch et al., 2000), thus giving rise to a total solid concentration in the autotrophic granular reactor of 5000 g-COD/m³. As provided in Table S5 (SI), diffusion of soluble components in the granule matrix was set with the introduction of reduced diffusion coefficients in water, while zero-flux of soluble components was considered at the granule center. Similar to the previous comparative studies (Chen et al., 2019; Sabha et al., 2017; Sabha et al., 2015), the mass transfer resistance through the liquid boundary layer was neglected for all soluble components, meaning that the concentration at the granule surface was identical to that in the bulk liquid. The steady-state granule size was controlled by the surface detachment velocity reported in Ni and Yuan (2013) without considering re-attachment of detached solids. For all the model simulations, venting of the produced N₂O was assumed to be achieved in the continuous outflow leaving the autotrophic granular reactor as well as through liquid-to-gas transfer of N₂O, which was modelled by incorporating a gas-liquid mass transfer equation (R_{N_2O}) mediated by the mass transfer coefficient (K_La) and the Henry's law constant (H) (Schulthess and Gujer, 1996) into the bulk liquid of all the different compartments.

$$R_{N_2O} = K_L a_{N_2O} (S_{N_2O} - \frac{S_{N_2O,air}}{H_{N_2O}})$$

where $K_L a_{N_2O}$ was assumed at 20 d⁻¹, $S_{N_2O,air}$ was 0.0003 g-N/m³, and H_{N_2O} was 1.9 (Chen et al., 2019).

All the model simulations were run to reach steady-state conditions indicated by constant effluent concentrations, N₂O production, granule size, and microbial composition. The main indexes used to evaluate the autotrophic granule reactor performance were the TN removal

efficiency (the percentage ratio of dinitrogen formed to influent ammonium) and the N_2O production factor (the percentage ratio of N_2O formed to ammonium removed). It should be noted that when calculating the N_2O formed, both the N_2O leaving the system together with the outflow and the N_2O being transferred to the atmosphere were taken into account.

3. RESULTS

3.1. Impact of Granule Size Distribution on TN Removal and N_2O Production in Autotrophic Granular Reactor

The impact of granule size distribution in the form of a varied number of granule size classes considered (i.e., Scenarios 1 to 5 in Table 1) on the autotrophic granule reactor was assessed in terms of the treatment performance (i.e., TN removal and N_2O production) and the composition of microorganisms and their associated contributions to N_2O production, with results demonstrated in Fig. 2. When the granules are of a uniform size (i.e., only 1 granule size class in the reactor), AOB (7%), Anammox bacteria (91%), and HB (2%) jointly dominate the active biomass in the autotrophic granules, leading to a TN removal efficiency of 90.1% (Fig. 2A). The increase in the number of granule size classes to 2 brings no significant change to the active microbial community structure and therefore leads to a nearly unchanged TN removal efficiency of 89.8%. However, when the extent of granule size distribution intensifies by increasing the number of granule size classes to 3, the abundance of HB decreases to 1%, while that of Anammox bacteria increases to 92%. Although the abundance of AOB remains almost stable at 7%, the corresponding TN removal efficiency drops to 88.7% (Fig. 2A). Further increasing the number of granule size classes to 4 and 5 tends to wash HB out of the reactor, rendering the abundance of HB of lower than 0.1%; AOB (7%) and Anammox bacteria (93%) are therefore the dominant contributing microorganisms in the autotrophic granules. The resultant TN removal efficiency decreases to

around 87.0%. As shown in Fig. 2A, despite the variation in the number of granules size classes considered, there is always no presence of NOB in the autotrophic granules.

Comparatively, a distinct trend has been observed in Fig. 2B for the N_2O production dynamics with respect to the number of granule size classes. On the condition of 1 granule size class, the lowest N_2O production factor is achieved at 1.8%. Judged by their contributions to N_2O turnover, AOB result in N_2O formation while HB lead to N_2O consumption. When the extent of granule size distribution is still low with the number of granule size classes of 2, the N_2O production factor slightly increases to 1.9%, due to the less than 1% exchange between the contributions of AOB and HB to N_2O turnover (Fig. 2B). Further increasing the number of granule size classes diminishes the negative contribution of HB to N_2O production, hence the rising N_2O production factor. At the number of granule size classes of 4, the N_2O production factor reaches the maximum value of 2.9%, with less than 1% negative contribution of HB to N_2O turnover. The highest extent of granule size distribution at the number of granule size classes of 5 only further reinforces the deprivation of the negative contribution of HB to N_2O production without significantly affecting the full N_2O production by AOB. Consequently, the N_2O production factor stays around 2.9% (Fig. 2B).

3.2. Profiles of Solid Components and N_2O Production Rates in Granules of Different Sizes

The detailed characteristics of granules in each granule size class of the autotrophic granular reactor considering a varied number of granule size classes were generated to assist the interpretation of the overall impact of granule size distribution on the autotrophic granular reactor. Fig. 3 delineates the fraction profiles of the 6 solid components in each granule size class, while Fig. 4 presents the corresponding profiles of relevant N_2O production rates

associated with AOB and HB as N_2O mediators. For the sake of easy presentation in Fig. 4, “r” refers to the rate of individual N_2O production process, with subscript denoting the N_2O -related conversion process and superscript denoting the contributing microorganism, while “R” indicates the net N_2O production rate mediated by certain contributing microorganism specified by superscript. A positive “r” or “R” represents N_2O production while a negative “r” or “R” means N_2O consumption.

3.2.1. Profiles of solid components in different granule size classes

In size class 1/1 (Fig. 3A), AOB and HB only exist at the granule surface, with fractions decreasing towards the granule center. The same trend has been observed for inert organics (X_I), the formation of which is mediated by HB. As a result, towards the granule center, the dominant fraction of Anammox bacteria first increases and then decreases due to the emerging presence of slowly biodegradable organics (X_S) in the inner part of granules. When granule size distribution is considered, significant differences exist between reactors with different numbers of granule size classes as well as between different granule size classes within the same reactor, as exhibited in Figs. 3B-3O. For the granular reactor with 2 granule size classes, Anammox bacteria solely dominate the inner part in size class 1/2 (Fig. 3B), while they occupy the inner part with the considerable joint presence of X_S in size class 2/2 (Fig. 3C). The fraction of HB, which coexist with AOB and Anammox bacteria at the granule surface, is much higher in size class 1/2 than size class 2/2. The profiles of solid components in size classes 1/3 (Fig. 3D) and 2/3 (Fig. 3E) are similar to those observed in size classes 1/2 (Fig. 3B) and 2/2 (Fig. 3C), respectively. The major difference in size class 3/3 from the aforementioned granule size classes lies in the disappearance of HB from the granules, compensated by a significant fraction of X_S at the granule surface (Fig. 3F). For the granular reactor with 4 granule size classes, the profiles of solid components in size class 1/4 (Fig. 3G)

are analogous to those in size classes 2/2 (Fig. 3C) and 2/3 (Fig. 3E), where a small fraction of HB exists at the granule surface. Nevertheless, the profiles of solid components in size classes 2/4 to 4/4 (Figs. 3H-3J) are comparable to each other and resemble those in size class 3/3 (Fig. 3F), where HB are absent from the granules. When the number of granule size classes reaches 5, all the granule size classes exhibit comparable trends regarding the fraction profiles of solid components (Figs. 3K-3O). These profiles are similar to those in granule size classes with relatively large granule sizes in the reactor with 3 or 4 granule size classes, e.g., size class 3/3 (Fig. 3F) and size classes 2/4 to 4/4 (Figs. 3H-3J). It should be noted that NOB are not able to grow in the autotrophic granule reactor under the operational conditions studied, regardless of the extent of granule size distribution.

3.2.2. Profiles of N_2O production rates in different granule size classes

As demonstrated in Fig. 4A, N_2O production only takes place at the granule surface where AOB and HB coexist in size class 1/1. The net N_2O production by AOB (i.e., the positive) is derived from the joint contribution of both the AOB denitrification pathway () and the NH_2OH pathway (), the latter of which possesses a higher rate. On the contrary, the net N_2O consumption by HB (i.e., the negative) is ascribed to the significant rate of N_2O reduction to N_2 (), compared to the marginal rate of N_2O formation from NO (). The most distinct difference between size classes 1/2 (Fig. 4B) and 2/2 (Fig. 4C) lies in the rate of N_2O reduction to N_2 () which to the most degree determines the N_2O consuming capability of HB (i.e.,): the magnitude of $r_{N_2O \rightarrow N_2}^{HB}$ is much higher in size class 1/2 (Fig. 4B) than size classes 2/2 (Fig. 4C). Similar to the trends about solid components observed in Section 3.2.1, the profiles of N_2O production rates in size classes 1/3 (Fig. 4D) and 2/3 (Fig. 4E) are comparable to those revealed in size classes 1/2 (Fig. 4B) and 2/2 (Fig. 4C), respectively. In size class 3/3 (Fig. 4F), N_2O is solely produced by AOB; HB stand no contribution to N_2O

production/consumption due to their washout from the granules (Fig. 3F). For the reactor with 4 granule size classes, AOB play a dominant role in N_2O production accompanied by trace N_2O consumption by HB in size class 1/4 (Fig. 4G), while AOB are solely responsible for N_2O production via both pathways without any contribution by HB due to their absence in size classes 2/4 to 4/4 (Figs. 4H-4J). As shown in Figs. 4K-4O, in the reactor with 5 granule size classes, all the granule size classes without the presence of HB display comparable trends in terms of relevant N_2O production rates, similar to size class 3/3 (Fig. 4F) and size classes 2/4 to 4/4 (Figs. 4H-4J).

3.3. Role of Each Granule Size Class in N_2O Production in Autotrophic Granular Reactor

Fig. 5 was generated to quantify the contribution (in percentage) of each granule size class of the granular reactor with 2 to 5 granule size classes to the net N_2O production together with the associated contribution of each N_2O production pathway to N_2O turnover in each size class. In the reactor with 2 granule size classes (Fig. 5A), size class 1/2 is responsible for 20% of the N_2O production, with the AOB denitrification pathway, the NH_2OH pathway, and the HB pathway each accounting for 23%, 41%, and -36% of N_2O turnover. By comparison, size class 2/2 takes up the remaining 80% of the N_2O production, with the AOB denitrification pathway, the NH_2OH pathway, and the HB pathway each occupying 32%, 55%, and -13% of N_2O turnover. In the reactor with 3 granule size classes (Fig. 5B), size class 1/3 is responsible for 11% of the N_2O production, with 23%, 44%, and -33% N_2O turnover contributed by the AOB denitrification pathway, the NH_2OH pathway, and the HB pathway, respectively. In contrast, size class 2/3 takes up 41% of the N_2O production, with 32%, 58%, and -10% N_2O turnover coming from the AOB denitrification pathway, the NH_2OH pathway, and the HB pathway, respectively. The remaining 48% of the N_2O production is initiated by size class 3/3,

with 35% and 65% N_2O turnover contributed by the AOB denitrification pathway and the NH_2OH pathway, respectively, without the presence of the HB pathway. As demonstrated in Fig. 5C, size classes 1/4 to 4/4 contribute to 12%, 20%, 30%, and 38%, respectively, of the N_2O production. Except for size class 1/4 which involves -7% N_2O turnover from the HB pathway, only the AOB denitrification pathway (33%) and the NH_2OH pathway (67%) are effective in producing N_2O in size classes 2/4 to 4/4. In the reactor with 5 granule size classes (Fig. 5D), size classes 1/5 to 5/5 conduce to different N_2O production contributions (i.e., 7%, 13%, 21%, 27%, and 32%, respectively), despite the comparable N_2O turnover contributions by the AOB denitrification pathway (33%) and the NH_2OH pathway (67%) in the absence of the HB pathway in each granule size class.

To visualize the volumetric contribution of each granule size class to N_2O production, the volumetric ratio (in percentage) of each granule size class relative to the total volume of all granule size classes in its respective reactor was calculated and has been added on to Fig. 5. For the reactor with 5 granule size classes free from the HB contribution (Fig. 5D), the volumetric ratio is lower than the N_2O production contribution in granule size classes with a relatively small granule size (i.e., size classes 1/5 to 3/5), while the volumetric ratio is higher than the N_2O production contribution in granule size classes with a larger granule size (i.e., size classes 4/5 and 5/5). The same trend has been noted for the reactor with 4 granule size classes (Fig. 5C), which only involves marginal negative N_2O production contribution by the HB pathway. When the number of granule size classes is reduced to 3 in Fig. 5B, the significant negative N_2O production contribution by the HB pathway dwarfs the N_2O production in size class 1/3, thus closing up the discrepancy between the volumetric ratio and the N_2O production contribution. However, size classes 2/3 and 3/3 still exhibit the pattern similar to Figs. 5C and 5D. When the number of granule size classes is further reduced to 2 in

Fig. 5A, the HB pathway plays a significant role in reducing N_2O production in both granule size classes. As a consequence, the volumetric ratio is higher than the N_2O production contribution in size class 1/2, while the volumetric ratio is lower than the N_2O production contribution in size class 2/2. This trend in Fig. 5A is opposite to the one observed for the reactor with 4 or 5 granule size classes (i.e., Figs. 5C and 5D).

4. DISCUSSION

Different from the common practice of assuming a uniform granule size, this work deals with the impact of granule size distribution on the autotrophic granular reactor performing PN/Anammox with focus on the related N_2O production. This incorporation of granule size distribution into the model enables the description of exchange of solutes between granules of different sizes and is particularly suitable for capturing the reality in reactors without well-distributed granule size. As indicated in Fig. 3, the microbial community structure is significantly influenced by the granule size. In a granular reactor with granule size distribution, granules of different sizes (i.e., different surface/volume ratios) are subject to different ammonium loadings as well as different oxygen availability in the granular structure, which have also been documented by Volcke et al. (2012) when exploring the NOB activity in the one-stage PN/Anammox granular system. These differences govern the competition between HB and Anammox bacteria for nitrite and the competition between HB and AOB for oxygen in the confined space at the granule surface. Therefore, HB tend to be outcompeted in relatively large granules while being retained in a small quantity in smaller granules. This trend is intensified with the higher extent of granule size distribution until the number of granule size classes reaches 5, where HB are completely washed out of the granules. The discrepancies in microbial community structure lead to distinct N_2O production rates contributed by different N_2O production pathways in the granular structure of different

granule size classes (Fig. 4). The HB pathway tends to be effective in consuming N_2O in small granules, while the AOB denitrification pathway and the NH_2OH pathway make significant contributions to N_2O production in granules of different sizes. As a result, different granule size classes contribute differently to the net N_2O production in the autotrophic granular reactor (Fig. 5). In general, larger granules account for a higher portion of the net N_2O production in the autotrophic granular reactor. For an autotrophic granular reactor with a relatively high extent of granule size distribution (e.g., 4 or 5 granule size classes in this work), the volumetric contribution of smaller granules to N_2O production is higher than that of larger granules. Due to the N_2O production pathway differentiation mainly in the level of the HB pathway, the volumetric contribution of smaller granules decreases with the decreasing extent of granule size distribution. Therefore, the volumetric contribution of smaller granules to N_2O production is lower than that of larger granules in the autotrophic granular reactor with 2 granule size classes. The overall impact of granule size distribution in Fig. 1 shows that with the increasing extent of granule size distribution (i.e., the increasing number of granule size classes considered), the TN removal efficiency decreases consistently while the N_2O production factor increases until the number of granule size classes reaches more than 4. It needs to be emphasized that HB as the minor microbial species growing on microbial decay products have been shown to play an important role in regulating N_2O production in the autotrophic granular reactor with a varied extent of granule size distribution, while they have no significant impact on the TN removal, which is line with the simulation result reported by Mozumder et al. (2014).

The simulation-based findings obtained in this work have two meaningful implications for the autotrophic nitrogen removal granular reactor, although their validity can only be verified in the future, considering the current unavailability of experimentally documented correlation

between granule size distribution and N_2O production. Firstly, the ever-decreasing TN removal efficiency and the ever-increasing N_2O production factor with the increasing number of granule size classes (Fig. 1) indicates the necessity of keeping the granules as well-distributed as possible in order to obtain high TN removal whilst minimizing N_2O production. This might be done through proper hydrodynamic shear control (Bobade et al., 2018). A similar key role of granule size in affecting nitrogen removal as well as regulating N_2O production has been reported for an Anammox-based granular sludge (Zhu et al., 2018a). Secondly, when modelling N_2O production/emission from the autotrophic nitrogen removal granular reactor, granule size distribution should be taken into account. For an autotrophic granular reactor with a significant extent of granule size distribution, the consideration of a uniform granule size in modelling might produce a N_2O production factor lower than its actual level. As shown in Fig. 1, the N_2O production factor at 1 granule size class is around 40% lower than that at 5 granule size classes. Considering the significant global warming potential of N_2O , such a discrepancy will substantially alter the reliable prediction of the carbon footprint of the autotrophic granular reactor studied. However, in that case, a total of 4 distinct granule size classes to be considered in the model should be enough to produce the reliable N_2O production and TN removal, as the continuous increase in the number of granule size classes to 5 only causes less than 0.5% difference but increases the computational expenses exponentially (see Fig. S3 in the SI). Moreover, both the TN removal efficiency and the N_2O production factor remain unchanged with the further increase in the number of granule size classes to above 5 (data not shown). On the contrary, one might opt to a uniform granule size (i.e., one granule size class to be considered) when only targeting at the overall nitrogen removal performance of the autotrophic granular reactor, even though the actual size distribution might reflect the reality more closely. This is in view of the minor impact of granule size distribution on the TN removal efficiency (less than 4% change between 1 and 5

granule size classes in Fig. 1). This phenomenon is consistent with the finding reported in Volcke et al. (2012), despite the fact that the metabolisms of HB were not accounted for in their work.

It should be noted that the simulated results presented in this work are specific to the model structure and parameter set applied, which were verified in our previous work (Chen et al., 2019). This is inevitable on the premise that there is no generally accepted model which could be used to describe multiple N_2O production pathways. Even though the model has been shown to be capable of predicting the overall N_2O production (Chen et al., 2019), its credibility of differentiating N_2O production pathways remains to be further validated, which will rely significantly on the availability of reliable in-situ tracing techniques applicable to the autotrophic granular reactor studied in this work. In spite of all this, the critical role of granule size distribution in affecting N_2O production/emission from the autotrophic nitrogen removal granular reactor is undeniable.

5. CONCLUSIONS

This work investigated the impact of granule size distribution on the autotrophic granular reactor performing partial nitrification and Anammox with focus on the N_2O production. Main conclusions include:

- HB growing on microbial decay products tend to be retained and contribute to N_2O consumption in relatively small granules, while AOB are mainly responsible for N_2O production via two pathways of AOB in granules of different sizes. Such heterogeneity in the granular structure disappears when the number of granule size classes considered reaches more than 4, where HB are completely outcompeted in the granules.

- Larger granules account for a higher portion of the net N_2O production, while the trend regarding the volumetric contribution of each granule size class changes with a varied number of granule size classes, due to the different contributions of relevant N_2O production pathways (with the HB pathway being the most decisive).
- With the increasing extent of granule size distribution, the nitrogen removal efficiency decreases slightly but consistently, whereas the N_2O production factor increases until the number of granule size classes reaches 4 or above.
- Practically, granules should be controlled as well-distributed as possible in order to obtain high nitrogen removal (i.e., an efficiency of around 90.0%) whilst minimizing N_2O production (i.e., a N_2O production factor of lower than 2.0%).
- When modelling N_2O production/emission from the autotrophic nitrogen removal granular reactor, granule size distribution should be considered carefully and specifically.

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Table and Figure Legends

Table 1. Features of reactors with different granule size classes implemented in this work

Figure 1. Configuration implemented in Aquasim to model granule size distribution (i.e., $n \geq 2$) as presented in this work.

Figure 2. Effect of number of granule size classes on (A) TN removal efficiency and active biomass fraction and (B) N_2O production factor and contribution of AOB and HB pathways to N_2O turnover.

Figure 3. Profiles of solid components in each size class of granular reactor with (A) 1, (B and C) 2, (D, E, and F) 3, (G, H, I, and J) 4, and (K, L, M, N, and O) 5 granule size classes.

Figure 4. Profiles of relevant N_2O production rates in each size class of granular reactor with (A) 1, (B and C) 2, (D, E, and F) 3, (G, H, I, and J) 4, and (K, L, M, N, and O) 5 granule size classes.

Figure 5. Volumetric ratio of each size class with its corresponding contribution to net N_2O production and associated contribution of each N_2O production pathway in each size class of granular reactor with (A) 2, (B) 3, (C) 4, and (D) 5 granule size classes.

Table 1. Features of reactors with different granule size classes implemented in this work

Scenario 1 Reactor with 1 granule size class		Size class 1/1				
	Compartment volume (m ³)	1				
	Number of granules	3.82x10 ⁸				
	Granule radius (μm)	500				
Scenario 2 Reactor with 2 granule size classes		Size class 1/2	Size class 2/2			
	Compartment volume (m ³)	0.33333333	0.33333333			
	Number of granules	1.91x10 ⁸	1.91x10 ⁸			
	Granule radius (μm)	420	560			
Scenario 3 Reactor with 3 granule size classes		Size class 1/3	Size class 2/3	Size class 3/3		
	Compartment volume (m ³)	0.25	0.25	0.25		
	Number of granules	1.27x10 ⁸	1.27x10 ⁸	1.27x10 ⁸		
	Granule radius (μm)	350	490	600		
Scenario 4 Reactor with 4 granule size classes		Size class 1/4	Size class 2/4	Size class 3/4	Size class 4/4	
	Compartment volume (m ³)	0.2	0.2	0.2	0.2	
	Number of granules	9.55x10 ⁷	9.55x10 ⁷	9.55x10 ⁷	9.55x10 ⁷	
	Granule radius (μm)	290	430	540	620	
Scenario 5 Reactor with 5 granule size classes		Size class 1/5	Size class 2/5	Size class 3/5	Size class 4/5	Size class 5/5
	Compartment volume (m ³)	0.16666667	0.16666667	0.16666667	0.16666667	0.16666667
	Number of granules	7.64x10 ⁷	7.64x10 ⁷	7.64x10 ⁷	7.64x10 ⁷	7.64x10 ⁷
	Granule radius (μm)	260	380	490	570	630

Highlights

- Impact of granule size distribution on autotrophic granular N_2O production was studied.
- Heterogeneity in granular structure weakens with extent of granule size distribution.
- HB washout due to elevated granule size distribution increases N_2O production factor.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5