ARTICLE

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The Granule Size Distribution in an Anammox-Based Granular Sludge Reactor Affects the Conversion—Implications for Modeling

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ABSTRACT: Mathematical models are useful tools to optimize the performance of granular sludge reactors. In these models, typically a uniform granule size is assumed for the whole reactor, even though in reality the granules follow a size distribution and the granule size as such affects the process performance. This study assesses the effect of the granule size distribution on the performance of a granular sludge reactor in which autotrophic nitrogen removal is realized through one-stage partial nitritation–anammox. A comparison is made between different approaches to deal with particle size distributions in one-dimensional biofilm models, from the use of a single characteristic diameter to applying a multiple compartment model. The results show a clear impact on the conversion efficiency of the way in which particle size distribution is modeled, resulting from the effect of the granule size on the competition between nitrite oxidizing and anammox bacteria and from the interaction between granules of different sizes in terms of the exchange of solutes. Whereas the use of a uniform granule size is sufficient in case only the overall reactor behavior needs to be assessed, taking into account the detailed granule size distribution is required to study the solute exchange between particles of different sizes. For the latter purpose, the application of the widespread software package Aquasim is limited and the development of dedicated software applications is required.

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Introduction

Granular sludge reactors are a type of biofilm reactors in which biomass is grown in the form of dense, fast-settling granules, resulting in compact systems which allow a high loading rate due to a large biofilm surface area in the reactor. These reactors are ideally suited for wastewater treatment and may be applied for, for example (simultaneous) COD, nitrogen, and phosphorus removal. Mathematical models are useful tools to gain process insight and to optimize the performance of granular sludge reactors (Batstone et al., 2004; de Kreuk et al., 2007; Matsumoto et al., 2010; Ni et al., 2009a,b; Vazquez-Padin et al., 2010; Xavier et al., 2007). One of the aspects to be dealt with in these models concerns the granule size, more specifically the diameter in case a perfectly spherical shape is assumed, as is the case in one-dimensional models (only considering radial gradients). Typically a uniform granule size is assumed for the whole reactor, even though it is acknowledged that in reality the granules follow a size distribution. The granule size determines the granule surface to volume ratio, an important parameter for mass transport of solutes in the granular biofilm, and thereby affects reactor performance. Therefore, the choice of this parameter has clear implications on the reliability of the modeling results.

Autotrophic nitrogen removal comprises partial nitritation and anaerobic ammonium oxidation (anammox) as consecutive reactions. Ammonium is oxidized to nitrite (the so-called *nitritation* reaction) by ammonium oxidizing bacteria (AOB), while further oxidation to nitrate by nitrite oxidizing bacteria (NOB) is prevented. During *partial* nitritation, only half of the ammonium is converted to nitrite. This reaction is typically followed by the combination of ammonium and nitrite to mainly nitrogen gas and little nitrate in a so-called anammox reaction. Combined partial nitritation–anammox processes result in substantial savings in aeration costs (up to 63%) and external carbon addition costs (up to 100%) compared to conventional nitrification–denitrification over nitrate, at the same time minimizing CO_2 emission and sludge production. A full-scale application of nitrogen removal from industrial wastewater through one-stage partial nitritation–anammox in a granular sludge reactor was described by Abma et al. (2010). This process further fits in innovative process schemes for energy-efficient treatment of municipal wastewater (Kartal et al., 2010).

The effect of granule size on autotrophic nitrogen removal in a granular sludge reactor has been revealed in previous work (Volcke et al., 2010) and is related in this study to the oxygen penetration depth. From the effect of the granule size on the performance of granular sludge reactors, the question arises to which extent the modeling approach to deal with the granule size distribution influences the simulation results and which modeling approach should be preferred. The latter issues form the main focus of this contribution. Numerical simulations are carried out for a case study concerning one-stage partial nitritation—anammox. A comparison is made between (1) the use of a single characteristic diameter, (2) weighing the simulation results for various characteristic diameters, and (3) using a multiple compartment model. Besides these different modeling approaches, the effect of the characteristic diameter (arithmetic mean, surface-area weighed mean, and volumetric mean) is assessed as well.

Materials and Methods

Autotrophic Granular Sludge Reactor Model

The one-dimensional biofilm model in this study has been implemented in the Aquasim software (Reichert, 1994), which follows the general multispecies biofilm model of Wanner and Gujer (1986). The model describes growth and endogenous respiration of AOB, NOB, and anammox bacteria. The total reactor volume (bulk liquid and biomass granules) is considered fixed at 400 m^3 (confined); spherical biomass particles (granules) are grown from an initial radius of 0.01 mm to a predefined steady-state granule radius (by defining the detachment rate $u_{de} = (L_F/L_F^{ss})^{10} \cdot u_{F,LF}$ if $u_{F,LF} > 0$ else $u_{de} = 0$; with L_F the actual granule radius, L_F^{ss} the steady-state granule radius and $u_{F,LF}$ the advective velocity of biofilm solid matrix at the granule surface) such that the reactor eventually contains 100 m^3 of particulate material, comprising both active biomass as well as inerts generated during endogenous respiration. The oxygen level in the bulk liquid is controlled at 0.5 $g\,O_2\,m^{-3}$; the reactor temperature and pH are assumed constant ($T = 30°C$, $pH = 7$). The bulk liquid has been assumed well-mixed, and external mass transfer limitation to the granules has been neglected. The reactor behavior has been simulated for an influent with an ammonium concentration of 500 $g\,N\,m^{-3}$ and a dissolved oxygen concentration of 8 $g\,O_2\,m^{-3}$, fed at a flow rate of 2,500 $m^3\,d^{-1}$. Simulations have been performed for several years of operation to assure steady-state conditions. The model stoichiometry, kinetics and parameter values are given by Volcke et al. (2010).

Particle Size Distribution in a Granular Sludge Reactor

The particle size distribution for a full-scale granular sludge anammox reactor has been characterized experimentally by Vlaeminck et al. (2010), resulting in 6 granule size classes i with relative abundance $RDF(i)$ (Table I). It was found useful for a part of our study to group the first three particle classes into a single class with particle size 0.1–1 mm, as these classes constitute only a small mass fraction in the reactor. This results in a total of 4 granule size classes included in the numerical model. For each particle class i, a surface area-weighed mean diameter $d_{msa}(i)$ and a volumetric mean diameter $d_{mvol}(i)$, corresponding to the mean surface area

Table I. Definition and characteristics of the particle size classes in this study.

Particle size class[a] (mm)	Relative abundance[a] $RDF(i)$ (%VSS)		Arithmetic mean diameter d_{mar} (mm)		Surf. area-weighed mean d_{msa} (mm)		Volumetric mean d_{mvol} (mm)	
0.1–0.25	2.5	10[c]	0.175	0.55[c]	0.1904	0.7106[c]	0.2026	0.7940[c]
0.25–0.50	1		0.375		0.3953		0.4127	
0.5–1	6.5		0.75		0.7906		0.8255	
1.0–1.6	35		1.3		1.334		1.366	
1.6–2	35		1.8		1.811		1.822	
2–3[b]	20		2.5		2.550		2.596	
Overall			1.55		1.765	1.767[c]	1.871	1.872[c]

[a]Vlaeminck et al. (2010).
[b]Assuming a maximum granule size of 3 mm—not determined by Vlaeminck et al. (2010).
[c]For a reduction to four particle size classes.

and granule volume in this class, respectively, are calculated from

$$\frac{1}{2}[\pi d^2_{\max}(i) + \pi d^2_{\min}(i)] = \pi d^2_{\mathrm{msa}}(i)$$

$$\frac{1}{2}\left[\frac{\pi}{6} d^3_{\max}(i) + \frac{\pi}{6} d^3_{\min}(i)\right] = \frac{\pi}{6} d^3_{\mathrm{mvol}}(i)$$

in which $d_{\min}(i)$ and $d_{\max}(i)$ represent the minimum and maximum diameters in class i, respectively. No maximum granule size was reported by Vlaeminck et al. (2010); this value was assumed 3 mm in this study. The overall surface area-weighed mean diameter, $d_{\mathrm{msa,overall}}$, and the overall volumetric mean diameter, $d_{\mathrm{mvol,overall}}$, for a number of classes ($n_{\mathrm{class}} = 4$ or 6) take into account the relative abundance $RDF(i)$ of each class and result from

$$\sum_{i=1}^{n_{\mathrm{class}}} RDF(i) \pi d^2_{\mathrm{msa}}(i) = \pi d^2_{\mathrm{msa,overall}}$$

$$\sum_{i=1}^{n_{\mathrm{class}}} RDF(i) \frac{\pi}{6} d^3_{\mathrm{mvol}}(i) = \frac{\pi}{6} d^3_{\mathrm{mvol,overall}}$$

In this case, given the low relative abundance of small particles, the overall surface area-weighed and volumetric mean diameters for four classes are about the same as for six classes (1.765 mm vs. 1.767 mm for $d_{\mathrm{msa,overall}}$; 1.871 mm vs. 1.872 mm for $d_{\mathrm{mvol,overall}}$). Table I summarizes the particle size characteristics. Note that a volumetric granule diameter equals the mass-weighed value, assuming the same particle density for all granule sizes. The overall arithmetic mean diameter has been calculated as well.

In this study, a comparison has been made between several granular sludge reactor models, differing in the particle size characterization:

- Uniform granule size, considering a single characteristic granule size equal to the overall arithmetic mean diameter $d_{\mathrm{mar,overall}} = 1.55$ mm, the overall surface area-weighed mean diameter $d_{\mathrm{msa,overall}} = 1.765$ mm or the overall volumetric mean diameter $d_{\mathrm{mvol,overall}} = 1.871$ mm.
- A class-weighed approach with six particle size classes, characterized by arithmetic mean diameter $d_{\mathrm{mar}}(i)$, surface area-weighed mean diameter $d_{\mathrm{msa}}(i)$, or volumetric mean diameter $d_{\mathrm{mvol}}(i)$ (Table I).
- A class-weighed approach with four particle size classes, characterized by $d_{\mathrm{mar}}(i)$, $d_{\mathrm{msa}}(i)$, or $d_{\mathrm{mvol}}(i)$ (Table I).
- A granule size distribution, four classes with relative abundance $RDF(i)$ and characteristic diameter $d_{\mathrm{mar}}(i)$, $d_{\mathrm{msa}}(i)$, or $d_{\mathrm{mvol}}(i)$ (Table I).

In the class-weighed approach, the simulation results (concentrations of ammonium, nitrite, nitrate, and nitrogen gas) obtained for the characteristic diameter (arithmetic mean, surface area-weighed mean, or volumetric mean) for each size class (4 or 6 simulation runs with uniform granule size) have been weighed according to the relative abundance of each class, being its relative contribution to the overall biomass volume, $RDF(i)$ (Table I). This class-weighed approach can be seen as a kind of simplified Monte-Carlo simulation, in which each class is represented by its characteristic diameter. It is important to note that the *interaction* between particles of different sizes in terms of the exchange of solutes is neglected in the class-weighed approach. Nevertheless, it is clear that a component (e.g., nitrite) produced in a granule of a certain size may be converted in a granule of another size, on its turn influencing the biomass composition in the granules and again the overall performance. This problem is overcome by considering different particle sizes in a single model (1 simulation run). In this way the size distribution becomes an integral part of the model. In Aquasim this can be realized by coupling as many biofilm compartments as size classes considered, each of which is characterized by a uniform granule size, $d_p(i)$ (e.g., the arithmetic mean, surface area-weighed mean or volumetric mean), while exchanging their bulk liquid at a sufficiently high rate to allow the same bulk composition in all compartments. This type of implementation has been demonstrated before by Morgenroth et al. (2000), coupling two biofilm compartments to evaluate two different characteristic biofilm regions.

With respect to the model considering a granule size distribution, choosing an appropriate model configuration, combined with setting the exchange flow rate between different compartments to an appropriate value was found to be a laborious task: a too low exchange rate resulted in different bulk concentrations between compartments, while a too high exchange rate may cause numerical problems and mass balance violations (such as the sum of all nitrogen component concentrations being higher than the incoming flow). For this reason, the number of size classes (biofilm compartments) considered has been limited to 4. Besides, a completely mixed compartment has been added. The total volume (granules + bulk liquid) of each compartment has been set such that all compartments have the same bulk volume (60 m^3), once the granules have grown to their full size. The feed flow rate has been divided over the biofilm compartments proportionally to the amount of biomass in each compartment. Recirculation flows have been added between every two compartments in the form of advective links with bifurcations (all having the same flow rate $Q_{\mathrm{ex}} = 10{,}000\ \mathrm{m^3\ d^{-1}}$). The resulting configuration, as depicted in Figure 1, yields satisfactory results: concentrations differences between compartments are lower than 1% of the incoming ammonium concentration, while no numerical problems are encountered—the nitrogen balance is closed.

Results

Influence of the Granule Size on the Reactor Performance

The influence of the granule size on the steady-state reactor performance was assessed in terms of the bulk

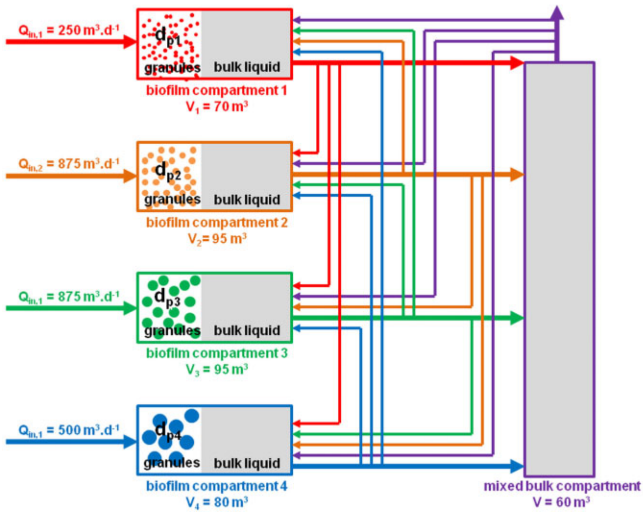

Figure 1. Implementation of the granular sludge reactor model with four granule size classes in Aquasim. Outgoing streams are modeled through advective links, of which bifurcations are denoted by thinner lines. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

concentrations of nitrogen compounds (Fig. 2A) and biomass and inert fractions in the granules (Fig. 2B). In small granules, nitrate is produced by NOB. Anammox activity increases as the granule size increases, which is reflected by an increased nitrogen gas production and an increased anammox biomass fraction in the granule (even though it is expected that the latter will again decrease for even larger particles, in favor of inert material). For large granules, anammox bacteria completely outcompete NOB—the nitrate formed then only results from the anammox conversion. For intermediate granule sizes, not all nitrite produced can be further converted to nitrate (by NOB) or to nitrogen gas (by anammox bacteria), resulting in nitrite accumulation. Figure 2C displays the corresponding oxygen penetration depth, defined in this study as the zone for which the oxygen concentration is higher than $0.1\ \mathrm{g\,O_2\,m^{-3}}$, as well as the resulting anoxic volume fraction. For a granule size increasing from 0.79 to 2.55 mm, the oxygen penetration depth decreases from 0.06 to 0.02 mm, corresponding to an increasing anoxic fraction from about 60–95%. For smaller granules ($d_p \leq 0.55$ mm), the diffusion of ammonium is limiting rather than the diffusion of oxygen, for which the concentration does not fall below $0.1\ \mathrm{g\,O_2\,m^{-3}}$ throughout the granule.

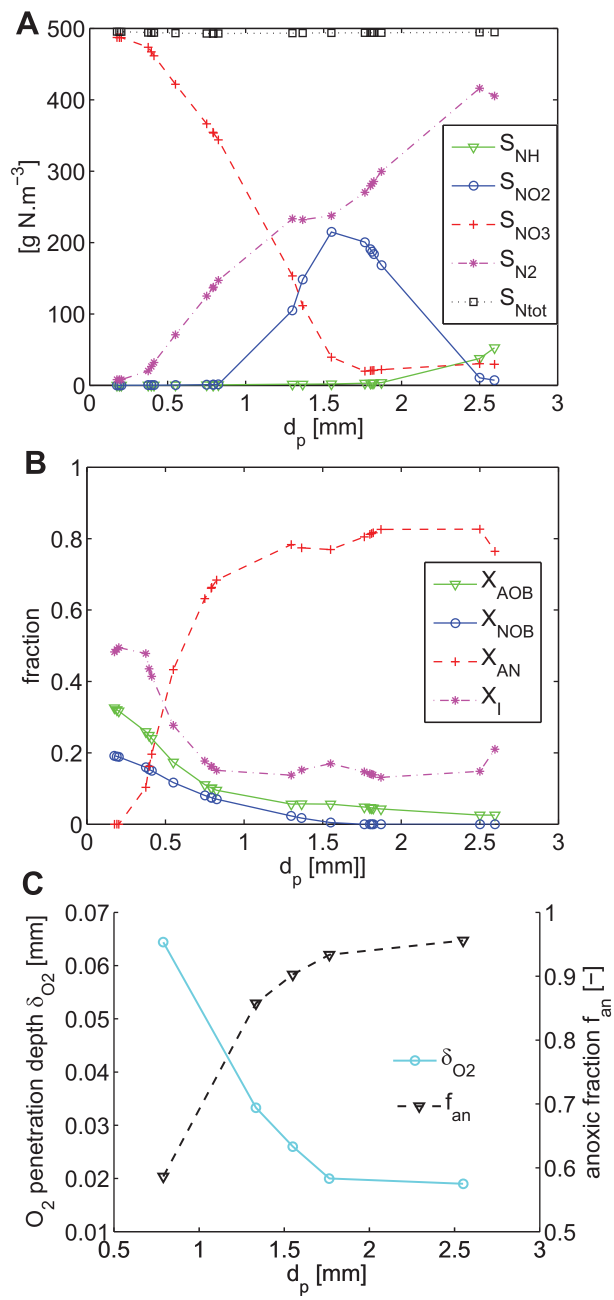

Figure 2. Influence of granule diameter d_p on steady-state reactor performance (simulation results obtained for a uniform granule size): (**A**) bulk concentrations of ammonium (S_{NH}), nitrite (S_{NO_2}), nitrate (S_{NO_3}), nitrogen gas (S_{N_2}) and their sum (S_{Ntot}); (**B**) biomass fractions (X_{AOB}: ammonium oxidizers; X_{NOB}: nitrite oxidizers; X_{AN}: anammox bacteria) and particulate inerts (X_I) in the granule; (**C**) oxygen penetration depth (δ_{O_2}) and anoxic volume fraction (f_{an}). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

Assessing the Reactor Performance for a Given Particle Size Distribution

The outcome of the different modeling approaches for the granular sludge reactor, differing in the way the particle size distribution is taken into account, is displayed in Figure 3. The results obtained with the class-weighed approach considering four classes are not taken up, since they are almost the same as with the class-weighed approach for six classes. This is not surprising seen the small "weight" of the first two (of six) classes. Nearly complete ammonium conversion was achieved with all modeling approaches (98–100%, results not shown). However, the simulation results show clear differences regarding the amount of ammonium converted into nitrite (21–43%), nitrate (4–22%), or nitrogen gas (48–60%).

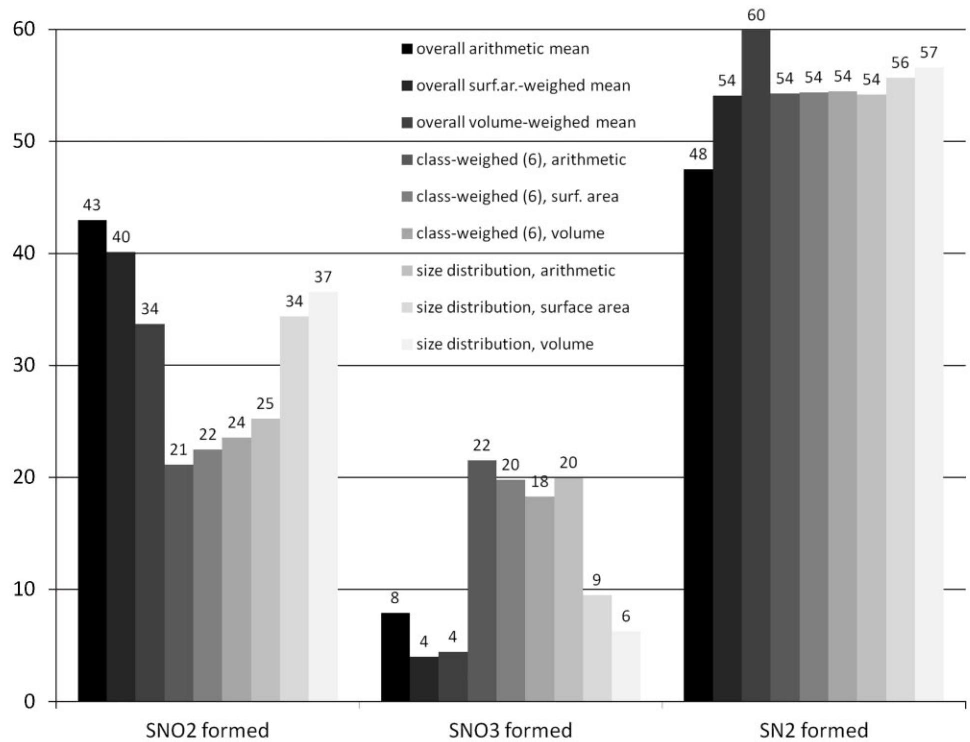

Figure 3. Simulated reactor performance in terms of nitrite (S_{NO_2}), nitrate (S_{NO_3}), and nitrogen gas (S_{N_2}) formation—comparison between different modeling approaches. The figures on the bars indicate the percentages of product formation.

On the one hand, for a given modeling approach (uniform granule size, class-weighed approach, or granule size distribution), the results differ according to the choice of the characteristic diameter. In particular, the amount of nitrate formed decreases as the characteristic diameter increases, from arithmetic mean over surface-area weighed mean to volumetric mean. Besides, among the three models with multiple granule size classes ("size distribution"), the simulation results obtained by characterizing each class by its arithmetic mean diameter are relatively far off from the ones based on the surface-area weighed or volumetric mean diameter.

On the other hand, given a characteristic diameter, no clear relation can be distinguished between the different modeling approaches. The results obtained with the class-weighed approach can be very different from the ones obtained by considering multiple granule sizes at once. For instance, using the surface-area weighed mean as a characteristic diameter, the reactor performance differs significantly between the class-weighed and the size distribution approach in terms of nitrite formation (22 vs. 34%, respectively) and nitrate formation (20 vs. 9%, respectively). Moreover, the simulation results obtained with a uniform particle size are closer to the ones taking into account the granule size distribution than to the ones using a class-weighed approach.

Influence of Size Distribution on Granule Composition

The importance of taking into account the particle size distribution is assessed by studying the distribution profiles for biomass and inert material inside the granules. Figure 4 displays the steady-state composition for the different granule sizes in the model taking into account the granule size distribution based on the surface area-weighed mean diameter $d_{msa}(i)$ of each class. Clearly distinct profiles are obtained for different granule sizes. NOB are only present in the smallest granules. As the granule size increases, relatively more inert material is present, at the expense of the active biomass fraction (AOB and anammox bacteria). Note that similar differences in the steady-state distribution of biomass and particulate inerts between granules of different sizes were obtained for the models taking into account the granule size distribution based on the arithmetic mean $d_{mar}(i)$ or the volumetric mean $d_{mvol}(i)$ as a characteristic diameter for each particle size class.

Discussion

Influence of Granule Size on Autotrophic Nitrogen Removal

Autotrophic nitrogen removal realized by one-stage partial nitritation–anammox in granular sludge reactors is

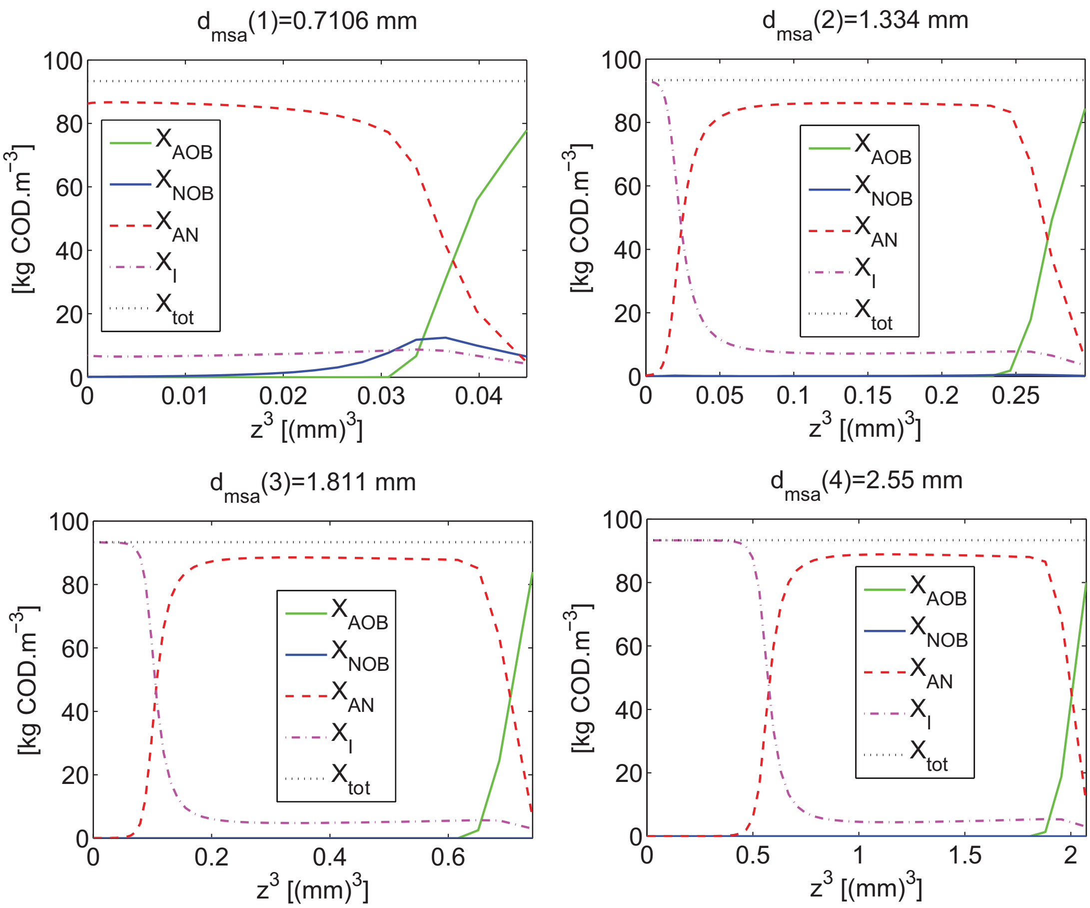

Figure 4. Steady-state distribution of biomass (X_{AOB}: ammonium oxidizers; X_{NOB}: nitrite oxidizers; X_{AN}: anammox bacteria), particulate inerts (X_I) and their sum (X_{tot}) in the granule along the distance *z* from the granule center. The profiles are shown in terms of z^3 to better represent volume fractions in the granule. Simulation results for granule size distribution based on surface area-weighed mean diameter $d_{msa}(i)$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

significantly influenced by the granule size. This simulation study clearly showed that for a given oxygen level and a given total biomass quantity in the reactor, the anammox conversion is favored in relatively large granules, while smaller granules tend to accumulate nitrite or even nitrate through the outcompetition of anammox bacteria by NOB. This behavior is in accordance with the experimental findings of Vlaeminck et al. (2010) based on aerobic and anoxic activity batch tests, describing a decreasing NOB activity with increasing granule size, nitrite accumulation for small aggregates, and autonomous nitrogen removal through partial nitritation–anammox in large granules. The influence of the granule size on the reactor performance results from the increasing ammonium surface load for increasing granule radius (lower surface/volume ratio). The simulation results (Fig. 2C) confirm that an increasing particle size and thus a larger ammonium surface load results in a smaller oxygen penetration depth and in this way in relatively less aerobic, and more anoxic volume. The latter holds despite the fact that the outside layer with a given thickness represents a larger volume for larger particles. As a result, anammox bacteria can accumulate to larger amounts in larger granules, giving them a competitive advantage over NOB (Winkler et al., 2011). Insight in the influence of granule size on the competition between NOB and anammox bacteria (besides the influence of the oxygen concentration, Hao et al., 2002) is critical for good process operation, in particular at lower temperatures, for the application of a one-stage cold anammox process (Kartal et al., 2010).

Evaluation of Different Modeling Approaches

A comparison was made between different approaches to deal with the particle size distribution in granular sludge reactor models. The modeling approach with multiple granules size classes in one model (Fig. 1) describes reality most closely in the sense that it takes into account the granule size distribution as well as the interaction in terms of solute exchange between granules of different sizes. The simulation results clearly show the presence of NOB in the smallest granules, while they are outcompeted by anammox bacteria in larger granules (Fig. 4). These results correspond to the experimental findings of Winkler et al. (2011), who revealed through the use of FISH and qPCR techniques that small granules are dominated by NOB bacteria and large granules by anammox bacteria. In the model taking into account the particle size distribution based on the arithmetic mean diameter, each class is represented by a relatively smaller granule size compared to the surface area-weighed or volumetric-weighed mean. As a result, more NOB are present in the smallest granules, which explains why there is significantly less nitrite and more nitrate accumulation when taking into account the particle size distribution based on the arithmetic mean diameter than when based on the surface area-weighed or volumetric-weighed mean diameter (Fig. 3). The results for the latter two lie closer together and are preferred based on their physical meaning (based on the mean bulk-granule exchange area and biomass volume, respectively). If a larger number of granule size classes would be chosen for the simulation with multiple compartments, the values obtained for the surface area-weighed mean diameter and for the volumetric mean diameter of a given class would even differ less. They would also be closer to the results obtained with the arithmetic mean as a characteristic diameter. As a result, in case the granule size distribution is taken up in detail (large number of granule size classes), it is less important which type of characteristic diameter is chosen for each class. However, in order to refine the granule size distribution by increasing the number of granule size classes, sufficiently detailed experimental data need to be available.

In case simulation with multiple size classes is not possible or too time-consuming, the class-weighed modeling approach, in which simulation results for different particles sizes are weighed according to the relative abundance of each class, was thought of as an alternative. It takes into account the granule size distribution, but neglects the interaction between granules within different size classes, in this case concerning the exchange of nitrite as an intermediate. The missing interaction between granules of different sizes in the class-weighed approach results in different bulk liquid concentrations of all nitrogen compounds for each granule size class, namely those of Figure 2. In contrast, in the size distribution approach, all granule size classes see the same bulk liquid concentrations, corresponding to the percentages of product formation in Figure 3. As the results obtained with the class-weighed approach can be very different from the ones obtained by considering multiple granule sizes at once, the interaction between granules of different sizes cannot be neglected. It is clear that the class-weighed approach is not a good modeling option.

In case only the overall reactor behavior needs to be assessed, one may still opt to use a single characteristic granule size, for reasons of simplicity. Indeed, even though incorporating the actual size distribution by including multiple granule size classes in one model describes reality most closely and will lead to detailed results in terms of the composition of granules of different sizes, such model will not necessarily lead to a better prediction of the overall reactor behavior. In case a single characteristic granule size is used, the overall surface-area weighed mean diameter or volumetric mean diameter should definitely be preferred over the overall arithmetic mean, because of their physical meaning: the surface area governs the exchange of substrates, while the volume determines the amount of biomass. No a priori preference is expressed for one of the two. Besides, it can reasonably be expected that the effect of inaccurate granule size distribution modeling can be compensated during the calibration of other model parameters, such as the affinity constants and inhibition constants of NOB and anmmox bacteria in the case of one-stage partial nitritation–anammox. Vice versa, once all other model parameters have been fixed, the granule diameter can be used as a calibration parameter, even though this means that the resulting value will not have a distinct physical meaning.

General Implications for Modeling Granular Sludge Reactors

The influence of the granule size on the performance of granular sludge reactors is not limited to one-stage partial nitritation–anammox processes. It will occur in all cases in which the availability of substrate or inhibiting components influencing the competition between different biomass species is affected by the granule size, such as for nitrification–denitrification (Su and Yu, 2006; Vazquez-Padin et al., 2010) or simultaneous N and P removal (de Kreuk et al., 2007). As the way in which the granule size distribution is described influences the simulation results, it is important to clearly state the applied modeling methodology. Typically a uniform granule size is assumed for the whole reactor even though it is usually acknowledged that in reality the granules follow a size distribution. For instance, Batstone et al. (2004) and Vazquez-Padin et al. (2010) characterize granules by their overall volumetric mean diameter, while de Kreuk et al. (2007) use a surface-mean diameter and Ni et al. (2009a, b) rely on an "average" diameter without specifying its nature. These simulation studies were all conducted with the software package Aquasim, which is still the most widespread simulation environment to simulate the behavior of various types of biofilm reactors (e.g., Lackner et al., 2008; Martina et al., 2010), including granular sludge reactors. However, the application of Aquasim to simulate multiple particle size classes at once was found limited by several numerical problems in this study. Therefore, dedicated software applications should be developed. An example of successful application of a granular sludge model with multiple size classes in Matlab has been reported by Su and Yu (2006).

Conclusions

- Numerical results indicate that autotrophic nitrogen removal realized as one-stage partial nitritation–anammox in granular sludge reactors is significantly affected by the granule size and its distribution.
- The anammox conversion is favored in relatively large granules, while smaller granules tend to accumulate nitrite or even nitrate. This is due to a smaller oxygen penetration depth and in this way relatively less aerobic, and more anoxic volume with increasing particle size.
- In a single granular sludge reactor, NOB are present in the smallest granules, while they are outcompeted by anammox bacteria in larger granules. Moreover, the interaction in terms of the exchange of solutes between granules of different sizes cannot be neglected.
- Using mathematical models to optimize the performance of granular sludge reactors—also for other than anammox-based processes, it is important to be aware of the fact that the way in which the granule size distribution is described, influences the simulation results. The adopted methodology should therefore be clearly stated, in particular regarding the nature of the characteristic diameter. For the latter, the surface-area weighed or volumetric mean diameters are good options.
- Whereas the use of a single characteristic diameter (uniform granule size) is sufficient in case only the overall reactor behavior needs to be assessed, taking into account detailed granule size distributions is required to study the solute exchange between particles of different sizes.
- The application of the widespread software package Aquasim for the simulation of one-dimensional biofilm models is limited in terms of taking into account detailed granule size distributions. For the latter purpose, the development of dedicated software applications is required.

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