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Effect of heterotrophic growth on autotrophic nitrogen removal in a granular sludge reactor

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This study deals with the influence of heterotrophic growth on autotrophic nitrogen removal from wastewater in a granular sludge reactor. A mathematical model was set-up including autotrophic and heterotrophic growth and decay in the granules from a partial nitritation-anammox process. A distinction between heterotrophic bacteria was made based on the electron acceptor (dissolved oxygen, nitrite or nitrate) on which they grow, while the nitrogen gas produced was 'labelled' to retrieve its origin, from anammox or heterotrophic bacteria. Taking into account heterotrophic growth resulted in a lower initial nitrogen removal, but in a higher steady state nitrogen removal compared with a model in which heterotrophic growth was neglected. The anammox activity is related with the fact that heterotrophs initially use nitrite as electron acceptor, but when they switch to nitrate the produced nitrite can be used by anammox bacteria. Increased anammox activity in the presence of heterotrophs, therefore, resulted in a marginally increased N_2 production at steady state. Heterotrophic denitrification of nitrate to nitrite also explains why small amounts of organic substrate present in the influent positively affect the maximum nitrogen removal capacity. However, the process efficiency deteriorates once the amount of organic substrate in the influent exceeds a certain threshold. The bulk oxygen concentration and the granule size have a dual effect on the autotrophic nitrogen removal efficiency. Besides, the maximum nitrogen removal efficiency decreases and the corresponding optimal bulk oxygen concentration increases with increasing granule size.

Keywords: anammox; biofilm; nitrification; modelling; numerical simulation; wastewater treatment

Introduction

Autotrophic nitrogen removal is an innovative technique for biological nitrogen removal from wastewater during which ammonium is nitrified to nitrite by ammonium-oxidizing bacteria (AOB) followed by subsequent oxidation of ammonium and reduction of nitrite to nitrogen gas by anammox bacteria. In this process, nitrification of nitrite to nitrate needs to be prevented by outcompeting nitrite-oxidizing bacteria (NOB), which can be achieved at relatively low oxygen levels.[1,2]

Heterotrophic organisms are a priori not expected when treating influent wastewater streams that contain only nitrogen and no organic carbon. However, heterotrophic bacteria can grow on microbial decay products and their presence may affect the process performance. Kindaichi et al. [3] described the coexistence of nitrifiers, i.e. AOB and NOB, and heterotrophs in a rotating disk reactor fed with an ammonium solution without organic matter. They demonstrated that there was an efficient food web (carbon metabolism) in the autotrophic nitrifying biofilm community, preventing the buildup of metabolites or waste materials of nitrifiers to significant levels. The significance of heterotrophic growth in nitrifying biofilm reactors fed with ammonium as the sole energy source was also studied by Nogueira et al. [4] and by Matsumoto et al. [5] for circulating bed biofilm reactors and granular sludge reactors, respectively, both through a combination of experimental work with numerical simulations. Anammox bacteria were not present in these systems. In a recent study, Ni et al. [6] investigated the interaction between anammox and heterotrophic bacteria in an anammox biofilm (without nitrifiers), of which the feed did not contain organic carbon. They found that the fraction of heterotrophs (23%) in the anammox biofilm was significantly lower than the heterotrophic fraction in nitrifying biofilms reported by Nogueira et al. [4] and Kindaichi et al. [3] (30–50%, respectively).

The effect of heterotrophs growing on influent organic substrate on the performance of a partial nitritation–anammox biofilm reactor was assessed by Hao and van Loosdrecht [7] for flat (planar) biofilms. In the latter simulation study, biomass decay was modelled through the endogenous respiration concept [8] and as such heterotrophic growth on biomass decay products was not considered. Lackner et al. [9] examined the effect of heterotrophic activity on completely autotrophic nitrogen

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removal in membrane-aerated (counter-diffusion) versus conventional biofilm systems (co-diffusion). They studied the influence of heterotrophs growing on decay products as well as on influent organic carbon.

In this contribution, the interaction between autotrophs and heterotrophs in granules capable of autotrophic nitrogen removal through partial nitritation-anammox was assessed for the first time. First, the effect of taking up heterotrophic growth in models for autotrophic nitrogen removal from wastewater streams in which only ammonium and no organic carbon is present is assessed. Both the dynamic and steady-state model behaviour are considered. Secondly, the influence of influent organic carbon on the autotrophic nitrogen removal efficiency is studied. Particular attention is paid to its implications for reactor operation in terms of the optimum bulk oxygen concentration to achieve maximum nitrogen removal efficiency. Finally, the effect of the granule size, being another parameter affecting the reactor performance, is investigated, with and without organic substrate present in the influent. While previous work [10,11] considered the influence of the bulk oxygen concentration and the granule size as individual effects, in this contribution the interaction between the granule size and the bulk oxygen concentration is examined as well.

Granular sludge reactor model

Process stoichiometry and kinetics

The one-dimensional model of Volcke et al. [10] for autotrophic nitrogen removal in a granular sludge reactor was extended to evaluate the effect of heterotrophic activity on the partial nitritation-anammox process. The stoichiometric matrix, kinetic expressions and model parameter values are summarized in the Supplementary Material (Tables S1–S3). Nitrification was described as a two-step process: oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by AOB (X_{AOB}), followed by nitrite oxidation to nitrate (NO_3^-) by NOB (X_{NOB}). Anammox bacteria (X_{AN}) convert ammonium and nitrite to nitrogen gas (N_2). Possible N_2O and NO emission by AOB during autotrophic nitrogen removal in granular sludge reactors as studied by Van Hulle et al. (see [12]) was not considered. Heterotrophic growth reactions were included using dissolved oxygen, nitrite (NO_2^-) or nitrate (NO_3^-) as an electron acceptor. As in most denitrification models,[13] a sequential denitrification mechanism has been assumed, in which nitrate is converted to nitrite and subsequently to nitrogen gas. This implies that the released intermediate nitrite is available for uptake by other bacteria besides heterotrophs, in this case NOB and anammox bacteria. The possible nitrite release by heterotrophic bacteria was recently also hypothesized by Winkler et al. [14] Based on batch tests results with heterotrophic aerobic granular sludge, Winkler et al. [14] suggested that denitrifiers reduced nitrate to nitrite supplying additional nitrite for NOB, leading to a higher NOB/AOB ratio in aerobic granular sludge than theoretically expected (nitrite loop theory).

Table 1. Competition (indicated by '+') between the autotrophic and heterotrophic organisms for substrate.

				X_H		
Substrate	X_{AOB}	X_{NOB}	X_{AN}	X_{H,O_2}	X_{H,NO_2}	X_{H,NO_3}
O_2	+	+		+		
NH_4^+	+	(*)	+	(*)	(*)	(*)
NO_2^-		+	+		+	
NO_3^-						+

(*) Note that some NH_4^+ is taken up as a nitrogen source for biomass growth by all micro-organisms.

To identify the importance of the respective electron acceptors in heterotrophic growth, in the model an (artificial) distinction is made between aerobic heterotrophs (X_{H,O_2}), anoxic heterotrophs using nitrite (X_{H,NO_2}) and anoxic heterotrophs growing on nitrate (X_{H,NO_3}). It is important to note that this distinction is only made to trace back the electron acceptors used for heterotrophic growth. As in reality there is no distinction between heterotrophic groups based on their electron acceptors, the corresponding reaction rates are expressed in terms of total heterotrophs ($X_H = X_{H,O_2} + X_{H,NO_2} + X_{H,NO_3}$). Another particular feature of the model in this study is the 'labelling' of nitrogen gas based on its origin, i.e. its production through the anammox conversion (S_{N_2A}) or by heterotrophic denitrification (S_{N_2H}).

To simulate the production of organic materials during biomass decay, the death-regeneration concept was used instead of the endogenous respiration approach followed by Volcke et al. [10] The death-regeneration concept comprises the transition of living cells to substrate as well as a fraction of inert material, through the decay of microorganisms and/or hydrolysis.[15] In this study, decay is assumed to generate soluble organic substrate (S_S) directly rather than producing particulate organic substrate (X_S), which is subsequently hydrolysed to S_S. This approach reflects that decay rather than hydrolysis of X_S is the rate-limiting step.

The competition for oxygen, ammonium, nitrite and nitrate between the three types of autotrophic organisms and the heterotrophic bacteria present in the reactor results in complex interactions (Table 1). The presented mathematical model is a suitable tool for studying these complex interactions. Detailed process stoichiometry and rate expressions are listed in Tables S1 and S2 of the Supplementary Material.

Reactor configuration, simulation parameters and initial conditions

A one-dimensional biofilm model, only considering radial gradients in spherical biomass particles, was set up to describe the autotrophic and heterotrophic interaction in a granular sludge reactor. The model was implemented in the Aquasim software.[16] The reactor had a fixed total volume (bulk liquid + granules) of 400 m^3. Spherical biomass particles (granules) were grown from an initial radius of 0.10 mm to a predefined steady-state granule radius, r_p (0.75 mm by default, and then varied from 0.25 to 2.50 mm to examine the effect of granule size) such that the reactor eventually contains 100 m^3 of granules. The oxygen level in the bulk liquid was assumed to be perfectly controlled at a fixed value (chosen between 0.1 and 4 g O_2 m^{-3}), while the temperature was set at 30°C. The bulk liquid was assumed to be well mixed, and the external mass transfer limitations were neglected to simplify the evaluation of the simulation results.

Biomass granules are typically quite dense and rigid, meaning that particulate components were displaced only due to the expansion or shrinking of the biofilm solid matrix. Besides, the biofilm porosity was assumed constant ($\varepsilon_W = 0.75$) and the initial fractions of particulate components were $\varepsilon_{\mathrm{XAOB}}^{\mathrm{ini}} = 0.1$, $\varepsilon_{\mathrm{XNOB}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XAN}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XH}}^{\mathrm{ini}} = 0.05$, $\varepsilon_{\mathrm{XH,A}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XH,NO_2}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XH,NO_3}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XH}}^{\mathrm{ini}}/3$, and $\varepsilon_{\mathrm{XI}}^{\mathrm{ini}} = 0$. The density of autotrophic biomass and particulate inert material in the granules was set to 60,000 g VSS m^{-3},[17] corresponding to 80,000 g COD m^{-3} (for a typical conversion factor of 0.75 g VSS g^{-1} COD [8]). The density of the heterotrophs was taken as 20,000 g VSS m^{-3},[17] which is equivalent to 26,666 g COD m^{-3}. It was, however, found that changing the heterotrophic biomass density from 26,666 to 80,000 g COD m^{-3} did not significantly affect the bulk nitrogen concentrations (less than 2% difference on the steady-state bulk concentrations of NH_4^+, NO_2^-, NO_3^- and N_2, for default simulation parameter values and for a bulk O_2 concentration ranging from 0.10 to 2.00 g O_2 m^{-3}). To assess the impact of heterotrophic growth in the granular sludge reactor model, a number of simulations were carried out considering only autotrophic growth (i.e. the initial concentration of heterotrophs was replaced with inert material $\varepsilon_{\mathrm{XH}}^{\mathrm{ini}} = 0$; $\varepsilon_{\mathrm{XI}}^{\mathrm{ini}} = 0.05$).

The reactor behaviour was simulated for an influent containing 300 g N m^{-3} ammonium ($NH_{4(\mathrm{in})}$), fed with a flow rate of 2500 m^3 d^{-1}, which corresponds with an ammonium surface load of 1.875 g N m^{-2} d^{-1} (w.r.t. steady-state granule size). It was assumed that no nitrite or nitrate was present in the influent. To evaluate the effect of influent organic substrate on the reactor performance, its concentration (S_{Sin}) was varied from 0 to 1000 g COD m^{-3}. The initial concentrations of soluble compounds in the bulk liquid and biofilm were assumed equal to the influent concentrations.

Besides dynamic simulations, simulations have been performed for several years of operation to assure that steady state has been reached in bulk concentrations as well as in the biomass distribution profiles in the granules. Note that a steady state in the bulk concentrations is reached faster than steady state concerning the biomass profiles. Besides, the time needed to reach steady state depends on the process conditions (e.g. the bulk oxygen level). For instance, the time needed for the bulk concentrations to reach steady state is 406 days when the imposed dissolved oxygen concentration is 0.1 g O_2 m^{-3}, whereas this time is shortened to 240 days for 0.3 g O_2 m^{-3}, but further increases to 2400 days for 1.0 g O_2 m^{-3}. Besides, the initial conditions play a role as well, in the sense that steady state is reached faster as they are closer to the steady-state conditions. For instance, the time needed to reach steady state is shortened from 240 to 93 days by increasing the initial granule size from 0.10 to 0.75 mm (at a bulk oxygen concentration of 0.30 g O_2 m^{-3}) and is further shortened to 62 days by increasing the initial anammox biomass fraction in the granule to 0.10 instead of 0.05 (while $\varepsilon_{\mathrm{XAOB}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XNOB}}^{\mathrm{ini}} = \varepsilon_{\mathrm{XH}}^{\mathrm{ini}} = 0.05$). Because of the strong influence the initial conditions have, the time needed to reach steady-state reactor operation (in terms of bulk liquid concentrations and the biomass composition of the granules) may be significantly different from the one observed in practice. Therefore, the optimization of the reactor start-up period falls beyond the scope of this study.

Results

Effect of heterotrophic growth on nitrogen removal

A first series of simulations were performed for an influent containing only ammonium and no organic carbon. In this case, heterotrophic growth takes place on decay material only.

Dynamic reactor behaviour

The dynamic model behaviour without and with heterotrophic growth is compared in Figure 1, for a bulk oxygen concentration of 0.5 g O_2 m^{-3}. Figure 1(a) displays the N_2 production dynamics with and without heterotrophic growth, as well as the contribution of autotrophic N_2 production (besides heterotrophic N_2 production) in the latter case. Initially (days 100–600), the N_2 production is higher without heterotrophic growth than with heterotrophic growth. From day 600 on, a higher N_2 production is observed when taking into account heterotrophic activity, up to a somewhat higher N_2 production (200 g N m^{-3} versus 212 g N m^{-3}) at steady state. In case heterotrophic growth is considered, the share of autotrophic N_2 production to the total N_2 production gradually increases, from 74% at day 200, over 96% at day 600 up to completely (100%) autotrophic N_2 production at steady state.

Figure 1(b) shows the dynamics of ammonium, nitrite and nitrate with and without heterotrophic growth. It takes about 100 days till complete ammonium oxidation is realized. This is followed by a period of nitrite accumulation, along with the ingrowth of AOB (results not shown). From day 300 onwards, the nitrite concentration decreases due to the ingrowth of anammox bacteria (results not shown), which consume nitrite but also compete with AOB for ammonium, resulting in a ‘plateau’ in the nitrite

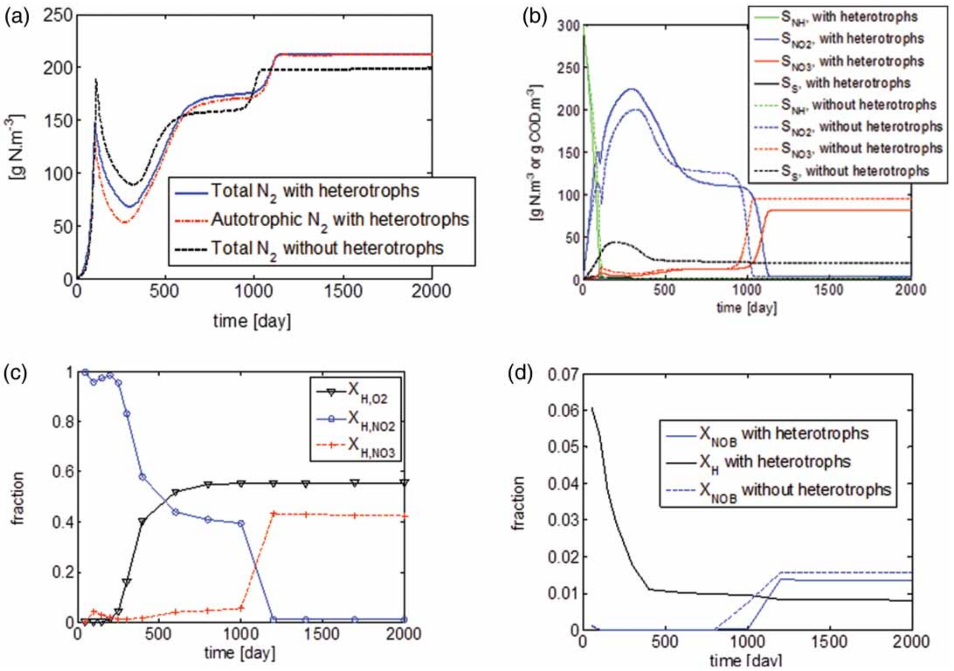

Figure 1. Comparison of dynamic model behaviour without and with heterotrophic growth in terms of (a) N_2 production dynamics and share of autotrophic N_2 production; (b) bulk concentrations of ammonium (S_{NH}), nitrite (S_{NO2}), nitrate (S_{NO3}) and soluble substrate (S_S); (c) fraction of heterotrophs growing on O_2, NO_2^- and NO_3^- as electron acceptor; (d) fraction of NOB and heterotrophic bacteria in a granule ($O_2 = 0.5\,g\,O_2\,m^{-3}$, $S_{Sin} = 0\,g\,COD\,m^{-3}$, $r_p = 0.75\,mm$, $NH_{4(in)} = 300\,g\,N\,m^{-3}$).

concentration. This phase is followed by a rapid drop of the nitrite concentration to around 2.50 g m^{-3} around day 1050 (without heterotrophs) – 1200 (with heterotrophs), due to the ingrowth of NOB (Figure 1(b)). A sharp increase in the nitrate formation takes place earlier (day 950 versus 1050) and the nitrate concentration reaches a higher level (95 versus 81 g N m^{-3}) without than with heterotrophic growth. This corresponds with an earlier ingrowth and a higher steady-state concentration of NOB (Figure 1(d)) in case heterotrophic growth is not considered. The concentration of soluble substrate (Figure 1(b)) follows the biomass decay rate (not shown) in case heterotrophic growth is not considered, while it is completely converted (resulting in a zero concentration) if heterotrophic growth is taken up in the model.

The dynamics of the heterotrophic community in terms of the electron acceptor it is consuming is shown in Figure 1(d). During the first phase, heterotrophs mainly grow on nitrite, which accumulates in the reactor until days 1000–1200 (Figure 1(b)). Once nitrite is depleted (days 1000–1200), heterotrophs switch to nitrate as an electron acceptor. From day 250 onwards, heterotrophic bacteria use oxygen as an electron acceptor as well, besides nitrite or nitrate.

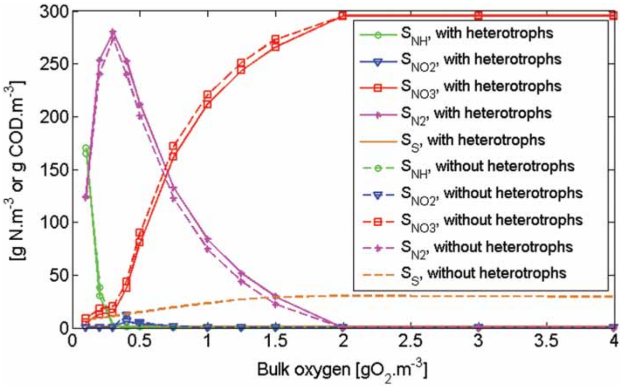

Figure 2. Influence of bulk oxygen concentration on steady-state reactor performance with and without heterotrophs ($r_p = 0.75\,mm$, $S_{Sin} = 0\,g\,COD\,m^{-3}$, $NH_{4(in)} = 300\,g\,N\,m^{-3}$).

Steady-state reactor behaviour

The steady-state reactor behaviour in terms of nitrogen removal as a function of the bulk oxygen concentration was compared for the cases with and without heterotrophic growth (Figure 2). Nitrogen gas is produced at low bulk oxygen concentration, while nitrate accumulates at high oxygen concentration. There is a clear peak of maximum nitrogen removal at 0.30 g O_2 m^{-3}in both cases. At this point, the total nitrogen production amounts to 280 g N m^{-3}, when heterotrophic growth is considered in the model and to 273 g N m^{-3} without heterotrophic growth, corresponding to 93% and 91% of the incoming ammonium, respectively. For oxygen concentrations below 2 g O_2 m^{-3} the nitrate (NO_3^-) accumulation is 1.5–3.5% higher without heterotrophs while at higher O_2 levels almost complete

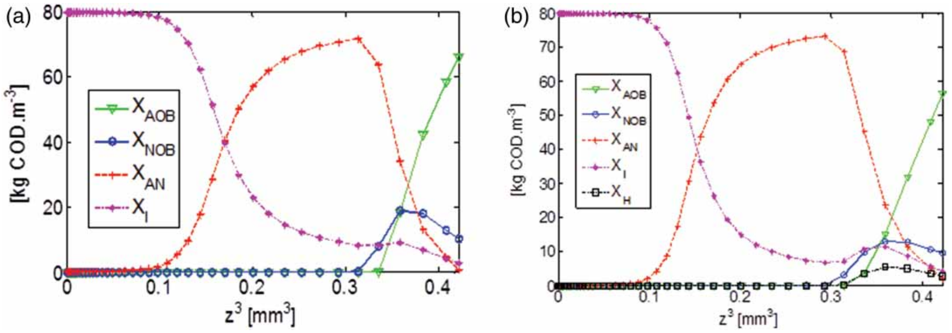

Figure 3. Steady-state distribution of biomass and particulate inerts in a granule (a) without taking up heterotrophic growth in the model and (b) with heterotrophs ($r_p = 0.75$ mm, $S_{Sin} = 0$ g COD m^{-3}, $NH_{4(in)} = 300$ g N m^{-3}, $S_{O2} = 1.0$ g O_2 m^{-3}. The profiles are shown in terms of z^3 to better represent volume fractions in the granule.

(98.5%) NO_3^- conversion is obtained in both cases. While there is no soluble substrate (S_S) accumulation in case heterotrophs are taken up in the model, without heterotrophs the S_S concentration increases from 8 to 30 g COD m^{-3} for increasing bulk oxygen concentrations from 0.1 to 2.0 g O_2 m^{-3}.

Typical steady-state biomass distribution profiles without and with considering heterotrophic growth in the model are displayed in Figure 3. AOB are located at the outside; their concentration is maximal at the surface of the granules. NOB and heterotrophic bacteria occupy the same position, just below the AOB. The anammox bacteria are located deeper in the granule, whereas inert material accumulates in the centre. It was found that the active biomass (X_{AOB}, X_{NOB}, Anammox and X_H) was located in a 0.3-mm band from the surface of the granule, independent of the steady-state granule radius (results not shown). Considering heterotrophic growth in the model results in a higher steady-state anammox fraction (43.5% versus 40.5%) and a lower steady-state NOB fraction (from 4.1% to 3.6%), besides heterotrophs (1.3%) in the granule (Figure 3). This corresponds with a higher N_2 production (83.5 g N m^{-3} versus 75.5 g N m^{-3}) and a lower NO_3^- production (211.6 g N m^{-3} versus 220.5 g N m^{-3}) in case heterotrophic growth is taken up in the model (Figure 2, for $S_{O_2} = 1\,g\,O_2$ m^{-3}).

Influence of influent organic substrate on reactor performance

In the simulations discussed in the previous section, no organic substrate was considered in the influent, so heterotrophic growth took place on decay products only. In a second series of simulations, the effect of (soluble, readily biodegradable) influent organic substrate (S_S) on the reactor performance was evaluated through the model in which heterotrophic growth was included.

The simulation results for a fixed bulk oxygen concentration (0.3 g O_2 m^{-3}, corresponding to the optimal value in Figure 2) and a changing influent organic substrate are summarized in Figure 4(a). For an increasing influent organic substrate concentration from 0 to 40 g COD m^{-3}, the N_2 production increased from 280 to 294 g N m^{-3} corresponding with 93% and 98% nitrogen removal, respectively, while the nitrate concentration decreased accordingly. For influent organic substrate concentrations between 40 and 60 g COD m^{-3}, the total N_2 production remained quite constant (around 98% N_2 production). However, as the influent organic substrate concentration was increased further, from 60 to 100 g COD m^{-3}, the N_2 production decreased from 292 to 244 g N m^{-3} (corresponding with 97% and 81% N_2 production), while ammonium started to accumulate. The nitrite production was negligible throughout the tested influent organic substrate concentration range.

The influence of the nitrogen removal performance for a fixed influent organic substrate concentration (100 g COD m^{-3}) with varying bulk oxygen concentration is displayed in Figure 4(b). The highest nitrogen removal efficiency (98%) is achieved in a bulk oxygen concentration range from 0.4 to 0.5 g O_2 m^{-3}.

A typical biomass distribution profile in the presence of influent organic substrate ($S_{Sin} = 100$ g COD m^{-3}) is displayed in Figure 5. The fraction of anammox bacteria, NOB and heterotrophs amounts to 55.5%, 2.24% and 5.86%, respectively. Compared with the case without influent organic substrate (Figure 3(b)), the presence of S_{Sin} implies not only a higher fraction of heterotrophs but also a higher anammox fraction and a lower NOB fraction, corresponding with a higher N_2 production (157 g N m^{-3}) and a lower NO_3^- production (136.8 g N m^{-3}) (Figure 4(b), for $S_{O_2} = 1$ g O_2 m^{-3}).

The combined effect of the influent organic substrate concentration and the bulk oxygen concentration is summarized in Figure 6, displaying the dependency of the maximum nitrogen removal efficiency (maximum N_2 production) and the corresponding optimum bulk oxygen concentration on the influent organic substrate concentration. While a small amount of influent organic substrate

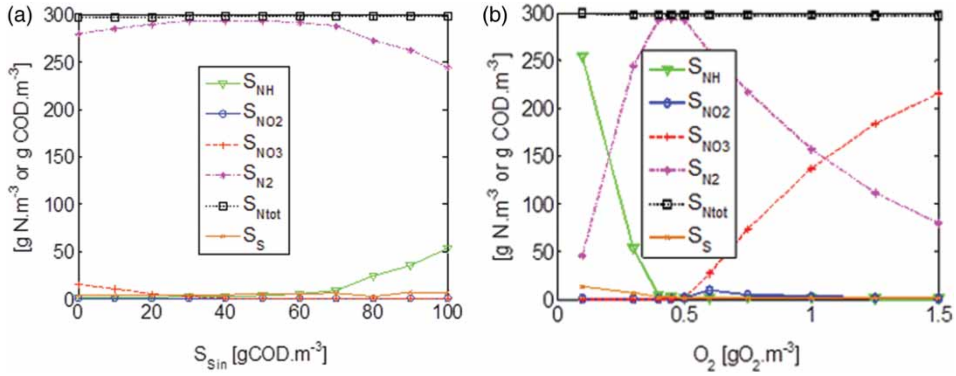

Figure 4. Steady-state reactor performance (a) influence of influent organic substrate (S_{Sin}) for a fixed bulk oxygen concentration ($S_{O_2} = 0.30\,g\,O_2\ m^{-3}$) and (b) influence of bulk oxygen concentration (O_2) for a fixed influent organic substrate concentration ($S_{Sin} = 100\,g\,COD\ m^{-3}$) ($r_p = 0.75$ mm, $NH_{4(in)} = 300\,g\,N\,m^{-3}$).

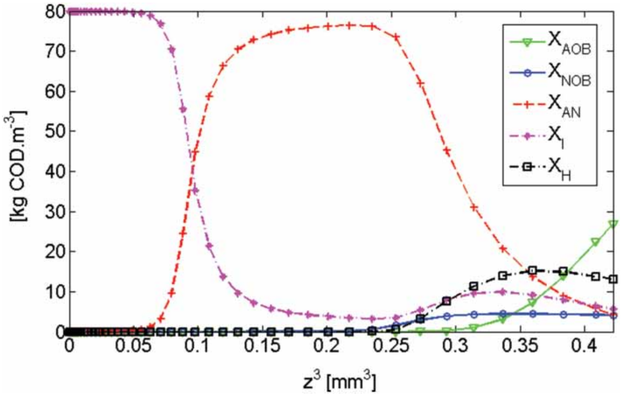

Figure 5. Steady-state distribution of biomass and particulate inerts in a granule in case organic substrate is present in the influent ($r_p = 0.75$ mm, $S_{Sin} = 100\,g\,COD\,m^{-3}$, $NH_{4(in)} = 300\,g\,N\,m^{-3}$, $S_{O_2} = 1.0\,g\,O_2\ m^{-3}$). The profile is shown in terms of z^3 to better represent volume fractions in the granule.

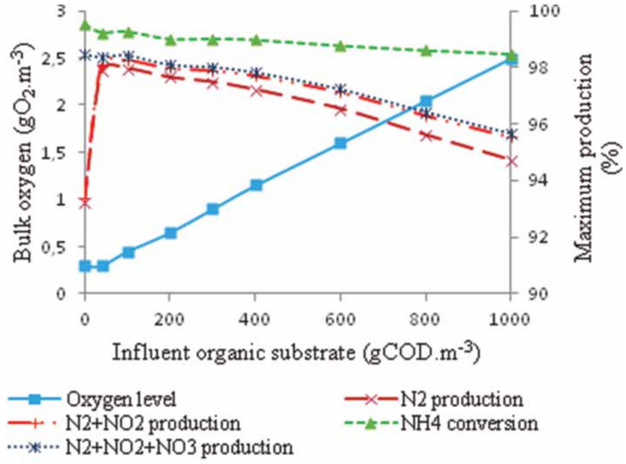

Figure 6. Influence of the influent organic substrate concentration on the maximum N_2 production, the corresponding ammonium removal efficiency, the nitrite and nitrate accumulation level, as well as the corresponding optimum bulk oxygen concentration ($r_p = 0.75$ mm, $NH_{4(in)} = 300\,g\,N\ m^{-3}$).

increases the maximum nitrogen removal capacity (from 93% without influent COD to 98% for $S_{Sin} = 40\,g\,COD\ m^{-3}$), the presence of larger amounts of organic substrate negatively affects the nitrogen removal capacity in terms of the N_2 production (down to 95% for $S_{Sin} = 1000\,g\,COD\ m^{-3}$). Besides, the necessary bulk oxygen concentration required to achieve this maximum N_2 production increases, from $0.3\,g\,O_2\ m^{-3}$ without influent COD to $2.5\,g\,O_2\ m^{-3}$ for $S_{Sin} = 1000\,g\,COD\ m^{-3}$. The ammonium conversion efficiency that corresponds with the maximum N_2 production level is almost the same (varies from 99.5% to 98.5%) over the whole influent organic substrate concentration range under study. The nitrite accumulation corresponding with maximum N_2 production is very small (from 0.2% for S_{Sin} lower than $40\,g\,COD\ m^{-3}$ up to 1% for $S_{Sin} = 1000\,g\,COD\ m^{-3}$). Nitrate production is negligible. The difference between the ammonium conversion and the production of N_2, NO_2^- and NO_3^- corresponds to ammonium incorporation in biomass. The latter fraction increases (from 1% to 3%) with increasing influent organic carbon concentration.

Influence of granule size on reactor performance

The influence of the granule size on the overall steady-state reactor performance in terms of nitrogen removal, at a fixed oxygen concentration ($1\,g\,O_2\ m^{-3}$), is compared in Figure 7 for the cases without and with organic substrate present in the influent. Without influent organic substrate, a maximal N_2 production of $261\,g\,N\ m^{-3}$, corresponding with 87% nitrogen removal, is observed for a granule radius of 2.00 mm. Any deviation from this point results in a lower nitrogen removal efficiency. For a decreasing granule radius, the N_2 production decreases (to 1.4% at 0.25 mm), at the expense of nitrate production. Besides, nitrite accumulates for granules with a radius between 1.00 and 2.00 mm, displaying a nitrite peak of 39.5% at a granule radius of

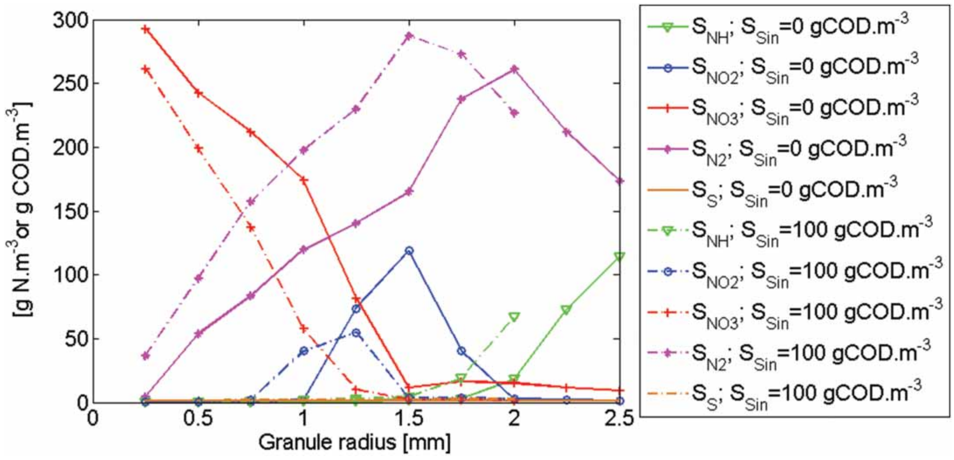

Figure 7. Influence of the granule size on the steady-state reactor performance for a fixed bulk oxygen concentration (solid lines for without organic substrate and dash lines for 100 g COD m^{-3} organic substrate at $S_{O_2} = 1.00$ g O_2 m^{-3}, $NH_{4(in)} = 300$ g N m^{-3}).

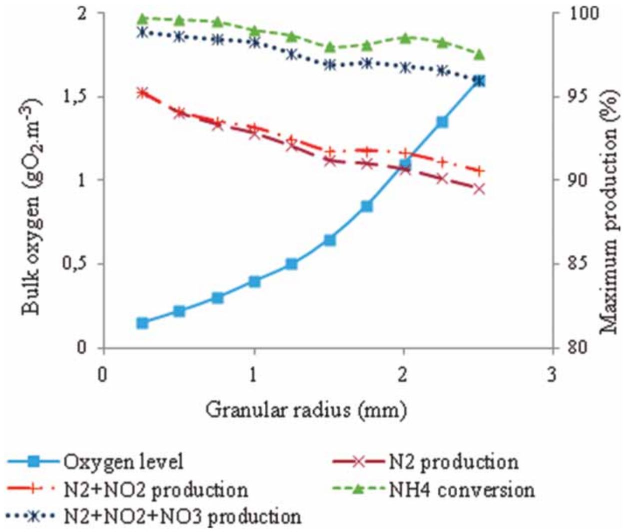

Figure 8. Influence of the granule size on the maximum N_2 production, the corresponding ammonium removal efficiency and nitrite accumulation level, as well as the corresponding optimum bulk oxygen concentration ($S_{Sin} = 0$ g COD m^{-3}, $NH_{4(in)} = 300$ g N m^{-3}).

1.50 mm. For larger granules, the decreasing N_2 production is associated with incomplete ammonium conversion.

In case the influent contains 100 g COD m^{-3} of influent organic substrate (S_{Sin}), the maximal N_2 production increases to 287 g N m^{-3} (95.7%) and is reached at a smaller optimal granule size (1.50 mm radius). Besides, there is less nitrite accumulation (peak of 18.2% at 1.25 mm).

The maximum N_2 production that can be obtained for each granule size, as well as the corresponding optimal bulk oxygen concentration, ammonium removal efficiency and nitrite accumulation level, is summarized in Figure 8. It is observed that the maximum nitrogen removal capacity (N_2 production) decreases with increasing granule size (from 95.3% to 89.5% for a granule radius from 0.25 to 2.50 mm).

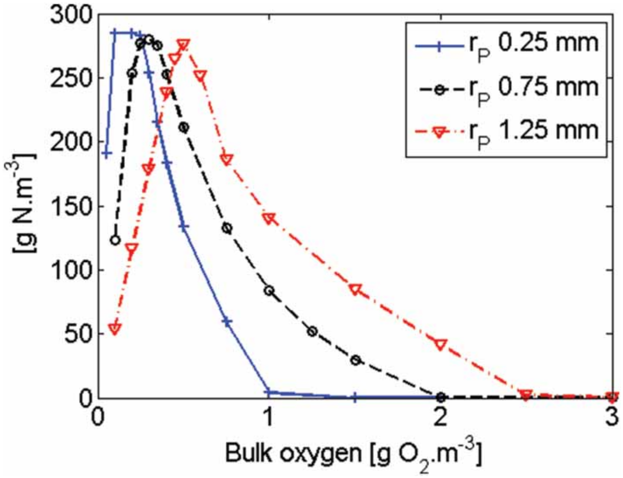

Figure 9. Steady-state N_2 production performance as a function of bulk oxygen concentration for different granule sizes ($S_{Sin} = 0$ g COD m^{-3}, $NH_{4(in)} = 300$ g N m^{-3}).

At the same time, the optimal bulk oxygen concentration required to achieve the maximum nitrogen removal increases, from 0.15 to 1.6 g O_2 m^{-3}, while the ammonium removal efficiency decreases slightly from 99.5% to 97.5%. The nitrite accumulation corresponding with maximum N_2 production is negligible (< 2.5 g N m^{-3}) throughout the investigated range of granule sizes.

A comparison between small, medium and large granules regarding the steady-state nitrogen removal (N_2 production) performance as a function of the bulk oxygen concentration is given in Figure 9. No straightforward conclusion can be drawn on the effect of the granule size on the broadness of the peak corresponding with maximum N_2 removal: The maximum N_2 removal efficiency is reached for a broader O_2 range in case of smaller granules; however, the peak itself seems broader for larger granules.

Discussion

Effect of the uptake of heterotrophic growth in models for autotrophic nitrogen removal

The usefulness of considering heterotrophic growth when modelling autotrophic nitrogen removal from wastewater containing only ammonium and no organic carbon was assessed in this study. It was shown that the uptake of heterotrophic growth on organic substrate originating from biomass decay in a model for autotrophic nitrogen removal results in a (slightly) higher steady-state N_2 production than in case heterotrophic growth is neglected (Figure 2). The optimal bulk oxygen concentration, at which the maximum N_2 production was obtained, was not affected by taking up heterotrophic growth in the model (Figure 2). Lackner et al. [9] compared through simulation the nitrogen removal efficiency with and without heterotrophs in membrane-aerated systems for autotrophic nitrogen removal in case no COD was present in the influent. They found no significant difference between scenarios for co-diffusion systems (O_2 and NH_4^+ diffuse from the same side – as is also the case for granules), whereas a significant negative impact (up to 40% lower nitrogen removal efficiency) of heterotrophs was determined for counter-diffusion systems (O_2 and NH_4^+ diffuse from opposite sides, e.g. membrane-aerated biofilm reactor).

Even though in this study the simulated reactor performance is only slightly affected by taking up heterotrophic growth in the model, the simulation results provide interesting insights into the interaction between the different functional groups in the granule. This was made possible by specific features of the model used in this study, namely the distinction of heterotrophic organisms based on their electron acceptor and N_2 labelling based on its origin. This type of methodology can also be applied to other process models, to study the growth mechanism of bacteria which can use various substrates or to identify the source of components which can be produced by multiple bacterial groups.

At steady state, all N_2 are produced by anammox bacteria, also in case heterotrophic growth is considered in the model (Figure 1(a)). This indicates that the additional N_2 production in the presence of heterotrophs is not due to the heterotrophs as such, but results from increased anammox activity in the presence of heterotrophs. This is explained by the dynamics of the heterotrophic community in terms of the electron acceptor it is growing on (Figure 1(c)). When growing granules from a small initial size, heterotrophs first compete with anammox bacteria for nitrite, which negatively affects the overall N_2 production (till day 600, Figure 1(a)). At steady state, heterotrophs switch to nitrate as the main electron acceptor (Figure 1(c)), which is reduced to nitrite and can be used by anammox bacteria, on its turn provoking an increased activity of the latter and resulting in a higher N_2 production and a lower nitrate production when taking up heterotrophic growth. Another reason for the higher N_2 production in the presence of heterotrophs lies in the competition of heterotrophs with NOB (besides AOB) for oxygen, which weakens the position of NOB as such (lower NOB fraction, see Figure 1(d)), and therefore also in the competition of NOB (X_{NOB}) with anammox (for nitrite), which constitutes a relative advantage for anammox bacteria.

Lackner et al. [9] explained the lower nitrogen removal efficiency for counter diffusion when considering heterotrophic growth by the competition between AOB and heterotrophs for oxygen, and by heterotrophs and anammox bacteria for nitrite. However, they did not perform a detailed analysis of heterotrophic growth, distinguishing between electron acceptors, as in this study. It should also be noted that the simulation results of Lackner et al. [9] relied on a model in which denitrification of nitrate was assumed to directly yield N_2, without intermediate nitrite production. The modelling assumption of parallel denitrification of nitrate and nitrite to N_2 implies that heterotrophs cannot release nitrite, which can be taken up by anammox bacteria (besides NOB), so a possible positive effect of heterotrophic growth on autotrophic nitrogen removal cannot be detected.

Influence of influent organic substrate

For a given bulk oxygen concentration, the steady-state nitrogen removal efficiency increases up to a certain level of influent organic substrate (Figure 4(a)). This is explained by the presence of heterotrophic bacteria that denitrify nitrate to nitrite – besides heterotrophic bacteria using oxygen as an electron acceptor. The produced nitrite is on its turn further denitrified to nitrogen gas by anammox bacteria. However, at higher influent organic substrate concentrations, the oxygen consumption by heterotrophic bacteria becomes so high that oxygen becomes limiting for ammonium oxidation, resulting in a lower N_2 production and in accumulation of ammonium (Figure 4(a)).

The maximum steady-state nitrogen removal performance that can be achieved, also increases for increasing, but relatively low ($< 40\,\mathrm{g\,COD\,m^{-3}}$) influent organic substrate concentrations and decreases for further increasing influent organic substrate concentrations (Figure 6). The positive effect of the presence of small amounts of organic substrate in the influent on the steady-state nitrogen removal performance (N_2 production) results from the presence of heterotrophic bacteria that denitrify nitrate to nitrite, which is on its turn further denitrified to nitrogen gas. While a pronounced effect of the influent organic substrate concentration on the maximum nitrogen removal efficiency and hardly any effect on the ammonium removal efficiency was found in this study (between 93% and 98% N_2 production in the range $S_{Sin} = 0 - 1000\,\mathrm{g\,COD\,m^{-3}}$, corresponding to a COD surface load of 0–6.25 $\mathrm{g\,COD\,m^{-2}\,d^{-1}}$; ammonium removal efficiency $99 \pm 0.5\%$), the effect of the influent organic carbon concentration on the N_2 production was not found significant (always around 90%, for a COD surface load between 0 and 12 g COD m^{-2} d^{-1}) by Hao and van Loosdrecht.[7] This can be explained by the fact that in the latter study, denitrification of nitrate was assumed to directly yield N_2, without intermediate nitrite production (i.e. parallel denitrification of nitrate and nitrite to N_2).

The optimal bulk oxygen concentration corresponding to the maximum nitrogen removal (N_2 production) performance increases with increasing influent organic substrate concentrations (Figure 6) because of the additional oxygen consumption by heterotrophic bacteria, as also found by Hao and van Loosdrecht.[7] In practice, a higher optimal bulk oxygen concentration implies higher aeration costs.

Hao and van Loosdrecht [7] found that the contribution of anammox to the (constant) total nitrogen removal gradually decreased and the heterotrophic nitrogen removal gradually increased with organic substrate surface load rate. In this study, it was found that the increasing optimal bulk oxygen concentration at higher influent organic substrate concentrations also promotes the ingrowth of NOB at the expense of anammox bacteria, resulting in nitrate accumulation and thus a lower maximum N_2 production efficiency with increasing influent organic substrate concentration.

Besides the positive effect of small influent organic substrate concentrations on the maximum N_2 production performance, the presence of organic substrate in the influent also results in a broader optimal oxygen range corresponding with maximum N_2 production (Figure 4(b)), in contrast to a narrow peak observed when treating influent only containing ammonium and no organic carbon (Figure 2). Hao and van Loosdrecht [7] also noted that the N_2 production peak becomes broader at increasing COD loading rates (for flat biofilms). As a result, the presence of organic substrate in the influent makes the process easier to control at its optimum bulk oxygen level. This is important since perfect oxygen control, exactly meeting the oxygen setpoint throughout the reactor, may be assumed during simulation (as in this study) but is difficult to realize in practice. These insights are beneficial for the application of anammox-based granular sludge reactors for nitrogen removal from municipal wastewater after the largest COD fraction has been removed, as in the scheme proposed by Kartal et al. [18]

Location of heterotrophic bacteria in a granule

The position of different microbial groups in a granule is determined by their competition for substrates and for space. The typical steady-state autotrophic biomass distribution profile inside granules capable of autotrophic nitrogen removal (Figure 3(a)) is known from earlier experimental [19] and simulation studies [10]: AOB are typically located at the outside of the granule; NOB (if present) are located just below the layer of AOB, while anammox bacteria are situated in the inner, anoxic part of the granule.

In this study, the position of heterotrophic bacteria in granules for autotrophic nitrogen removal was studied. In case no organic substrate is present in the influent, the maximum concentration of heterotrophic bacteria is found just below the layer of AOB (Figure 3(b)), as for the NOB. At this position, heterotrophs have easy access to O_2 (diffusing from the bulk liquid) and NO_3^- (produced by NOB) as their electron acceptors (Figure 1(c)), besides organic substrate originating from biomass decay and diffusing from both sides of the heterotrophs. For a co-diffusion (flat) biofilm treating exposed to a somewhat lower ammonium surface load (1.11 g N m^{-2} d^{-1}) and no organic substrate present in the influent, Lackner et al. [9] also found the largest fraction of heterotrophs close to the bulk liquid, in this case with a maximum concentration at the biofilm outside.

In case organic substrate is present in the influent (Figure 5, $S_{Sin} = 100$ g COD m^{-3}, corresponding with a surface load of 0.625 g COD m^{-2} d^{-1}), heterotrophic bacteria do not depend on biomass decay to get access to this substrate, which now also diffuses from the bulk liquid. As a result, the amount of heterotrophic bacteria present has increased compared with the case without influent organic substrate (Figure 5 versus Figure 3(b)), and the fast growing heterotrophs repress all other bacterial groups to a certain extent towards the inside of the granule (Figure 5). Besides heterotrophs, also the fraction of anammox bacteria increases, at the expense of inert materials. This is reflected in a higher (autotrophic) N_2 production and lower nitrite accumulation and nitrate production.

If the granules would be exposed to even higher influent organic substrate concentrations, it can be expected that the concentration of heterotrophic bacteria becomes maximal at the biofilm surface, as for the biomass distribution profiles obtained by Hao and van Loosdrecht [7] for flat autotrophic biofilms exposed to high COD surface loads (3–12 g COD m^{-2} d^{-1}) and a similar ammonium surface load (1.23 g N m^{-2} d^{-1}).

The presence of organic substrate in the influent does not compromise autotrophic nitrogen removal, as long as the volume of the biofilm (granules) is sufficiently large to contain all active biomass and still enough oxygen is provided to allow partial ammonium oxidation to nitrite (increasing optimum bulk oxygen concentration at increasing influent organic substrate concentrations, Figure 5) and in this way provide the substrates for the anammox conversion.

Duality and interaction between reactor oxygen concentration and granule size

It is well known that the performance of biofilm reactors for autotrophic nitrogen removal is strongly governed by the oxygen concentration in the bulk liquid, through its effect on the competition between anammox bacteria and NOB.[20] At very low oxygen concentrations, incomplete ammonium conversion takes place due to oxygen limitation, while anammox bacteria are outcompeted by NOB at high oxygen concentrations, resulting in nitrate accumulation. In between, there is an optimal bulk oxygen concentration at which the nitrogen gas production reaches a maximum. The range of optimal bulk oxygen concentrations is typically quite narrow, as is clear in Figure 2. A small deviation from the peak point corresponding with the optimal oxygen concentration results in a significant decrease in the nitrogen removal efficiency.

In case of a spherical biofilm, an increasing biofilm thickness results in an increasing ammonium surface load (lower surface/volume ratio), on its turn results causing a smaller oxygen penetration depth and in this way relatively less aerobic, and more anoxic volume.[10,11] As a result, the granule size is another key factor, besides the bulk oxygen concentration, which affects the nitrogen removal in a granular sludge reactor. Given a certain oxygen level – besides a given total biomass quantity in the reactor – there is an optimal granule size corresponding to maximum N_2 production (Figure 7). Smaller granules tend to accumulate nitrite or even nitrate, while the anammox conversion is favoured in larger granules, even though ammonium conversion is incomplete if the granules become too large. This behaviour was described by Volcke et al. [11] and is in accordance with the experimental findings of Nielsen et al. [21] Vlaeminck et al. [19] and Winkler et al. [22]

A kind of duality between the bulk oxygen concentration and granule size is noticed from the comparison of Figures 2 and 7, which are – in case no organic carbon is present in the influent – both characterized by a sharp optimum corresponding to the maximum N_2 production, while nitrite or nitrate is formed at higher bulk oxygen concentrations or for smaller granules and only incomplete ammonium conversion is realized at lower bulk oxygen concentrations or in larger granules. For a fixed granule size, the presence of organic substrate in the influent results in a higher N_2 removal efficiency at a higher optimal oxygen concentration (compare Figure 2 and Figure 4(b)). For a fixed bulk oxygen concentration, the presence of organic substrate in the influent results in a higher N_2 removal efficiency at a smaller optimal granule size (Figure 7). While the presence of organic substrate in the influent results in a broader range of optimal oxygen concentrations for a fixed granule size (Figure 4(b) compared with Figure 2), such broadening effect of influent organic carbon on the range of optimal granule sizes for a fixed oxygen concentration was not observed (Figure 7).

Whereas previous studies assessed the influence of the granule size at a fixed oxygen concentration, in this study the combined effect of the granule size and the bulk oxygen concentration was investigated as well. Indeed, the optimal bulk oxygen concentration corresponding to maximum N_2 production depends on the granule size and vice versa. It was demonstrated that the maximum N_2 production capacity decreases with increasing granule size at the expense of nitrate production, while the corresponding optimal bulk oxygen concentration increases and the ammonium removal efficiency slightly decreases (Figure 8). These simulation results contrast with the ones obtained for flat biofilms,[20] where the maximum nitrogen removal (N_2 production) efficiency increases with increasing biofilm thickness as long as the biofilm depth is limiting for anammox growth and efficiency remains constant for thicker biofilms. The decreasing maximum nitrogen removal capacity and increasing oxygen requirements with increasing granule size (Figure 8), for a comparable broadness of the peak expressing the N_2 removal efficiency in terms of oxygen (Figure 9) seems to imply that it is better to keep the granule size small. However, in practice the granules need to be large enough to ensure fast settling and easy biomass retention in the system.

Conclusions

- The heterotrophic uptake of organic substrate originating from biomass decay in a model for autotrophic nitrogen removal results in a (slightly) higher steady-state N_2 production by anammox bacteria than in case heterotrophic growth is neglected. This is due to the conversion by heterotrophic bacteria of nitrate to nitrite, which favours the growth of anammox bacteria.
- The modelling approach in which a single group of microorganisms was artificially split up according to the substrate used (in this case heterotrophic bacteria, based on the type of electron acceptor) and in which a single product was labelled depending on its origin (in this case heterotrophic or autotrophic N_2 production) proved useful to study the interaction between the present microbial groups.
- The maximum nitrogen removal capacity (N_2 production) in granules capable of autotrophic nitrogen removal through partial nitritation-anammox is improved by the presence of relatively small amounts of organic substrate in the influent, while larger quantities of influent organic substrate negatively affect the nitrogen removal capacity.
- The bulk oxygen concentration and the granule size have a dual effect on the autotrophic nitrogen removal efficiency, both showing a sharp optimum corresponding with the maximum N_2 production. For a fixed granule size (oxygen concentration), the presence of organic substrate in the influent results in a higher N_2 removal efficiency at a higher optimal oxygen concentration (at a smaller optimal granule size).
- The maximum N_2 production capacity decreases with increasing granule size at the expense of nitrate production, while the corresponding optimal bulk oxygen concentration increases and the ammonium removal efficiency slightly decreases.

Supplemental data

Supplemental data for this article can be accessed 10.1080/09593330.2013.859711.

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