Effect of granule size on autotrophic nitrogen removal in a granular sludge reactor

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Online publication date: 26 August 2010
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(Received 6 November 2009; Accepted 2 February 2010)

Autotrophic nitrogen removal through sequential partial nitritation and anammox reactions can be achieved in biofilm reactors by controlling the oxygen concentration in the bulk liquid in such a way that nitrite oxidizers are outcompeted by anammox bacteria. In the case of granular sludge reactors, the granule size may influence the optimal range of oxygen concentration, as has been confirmed in the present study by means of numerical simulations. The range of oxygen concentrations for which combined partial nitritation and anammox conversion is established becomes broader for larger particles and with increasing influent ammonium concentrations. At the same time the likelihood of nitrite accumulation in the reactor effluent also increases.

Keywords: anammox; biofilm; biological nitrogen removal; CANON; granular sludge; modelling; nitrification; OLAND; simulation

Introduction

Over recent decades biological processes for nitrogen removal from wastewater have proved their effectiveness and economic efficiency, and have become widely implemented to satisfy increasingly stringent effluent standards. Within the last few years innovative processes have been developed to improve the sustainability of biological nitrogen removal from wastewater. Many of these processes rely on the oxidation of ammonium to nitrite (the nitritation reaction), while further oxidation to nitrate is prevented. The nitrite produced can be directly denitrified to gaseous nitrogen, in which case the resulting process is described as nitrification–denitrification over nitrite. Another possibility is the conversion of only half the ammonium to nitrite (partial nitritation), followed by combination of ammonium and nitrite to form gaseous nitrogen in the 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\%) in comparison with conventional nitrification–denitrification over nitrate, at the same time minimizing the production of CO\textsubscript{2} and sludge.

Completely autotrophic nitrogen removal through partial nitritation–anammox can be achieved in separate reactors for the two reactions, such as in the SHARON–Anammox process, or in one-reactor configurations, termed aerobic/anoxic deammonification, CANON, OLAND, etc.; for an overview, see van der Star et al. [1]. In the one-reactor case the bacteria are usually grown in a biofilm configuration, with ammonium-oxidizing bacteria near the bulk liquid/biofilm interface providing oxygen for nitrification, and the anammox bacteria in the inner, anoxic part of the biofilm. Granular sludge reactors are a type of biofilm reactor 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 the large biofilm surface area in the reactor. Full-scale nitrogen removal through partial nitritation–anammox in granular sludge reactors has recently been demonstrated [2].

In order to successfully achieve completely autotrophic nitrogen removal through partial nitritation–anammox, it is essential to control the partial nitritation step in such a way that an optimal anammox nitrite:ammonium ratio is achieved. In a two-reactor SHARON–Anammox process, several control handles may be used to establish partial nitritation [3] independently of the anammox process. In one-step biofilm processes, on the other hand, careful regulation of the oxygen concentration in the bulk liquid is required, as has been demonstrated for flat biofilms using a numerical model [4]. An influence of the biofilm geometry on process performance is to be expected. Dealing with granular sludge reactors, a different particle radius implies a different surface:volume ratio, which may

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ISSN 0959-3330 print ISSN 1479-487X online
© 2010 Taylor & Francis
DOI: 10.1080/09593331003702746
http://www.informaworld.com
have an effect even if the total amount of biomass in the reactor remains unchanged. An influence of granule size has been observed in experiments and simulations by de Kreuk et al. for simultaneous N- and P-removal [5], and by Ni et al. in a simulation study of a stand-alone anammox process [6]. The influence of granule size on one-stage partial nitritation–anammox has so far only been assessed experimentally, by Vlaeminck et al. [7]. In the present study numerical simulations have been carried out to reveal the effect of granule size in the control of granular sludge reactors to obtain autotrophic nitrogen removal through partial nitritation–anammox. The influence of the influent ammonium concentration on the results has also been addressed.

**Autotrophic granular sludge reactor model**

**Reactor configuration: simulation parameters and influent conditions**

A one-dimensional biofilm model has been set up to describe the behaviour of the granular sludge reactor, considering only radial gradients. The model has been implemented using Aquasim software [8]. The reactor volume is assumed fixed at 400 m$^3$. Spherical biomass granules are grown from an initial radius of 0.01 mm to a predefined steady state granule radius $r_{gran}$ of 0.75, 1 or 1.5 mm, which are representative values found in a full-scale partial nitritation–anammox reactor. The number of granules was calculated such that the reactor full-scale partial nitritation–anammox reactor. The porosity has been assumed constant ($\varepsilon = 0.1$); $\varepsilon_{XAOB}^{ini} = \varepsilon_{XAN}^{ini} = 0.05$; $\varepsilon_{XIN}^{ini} = 0$). The biomass concentration in the granules is set to 70,000 g VSS m$^{-3}$, corresponding to 93,333 g COD m$^{-3}$ (for a typical conversion factor of 0.75 g VSS g$^{-1}$ COD, see Henze et al. [11]).

The reactor behaviour has been simulated for an influent containing mainly ammonium (300 g N m$^{-3}$), with a flow rate of 2500 m$^3$d$^{-1}$. In addition, the influent has also been assumed to contain a negligible amount of nitrite and nitrate (0.01 g N m$^{-3}$), in order to avoid numerical errors arising from zero concentrations in the kinetic expressions for the endogenous respiration rates $\rho_{ER}^{NO_2}$ and $\rho_{ER}^{NO_3}$. Table 1 presents the stoichiometric matrix for the completely autotrophic nitrogen removal process implemented in the present study. The corresponding process rates and stoichiometric and kinetic parameters are given in Tables 2 and 3, respectively.

The initial concentration of solutes in the bulk liquid has been assumed equal to the influent concentrations. Simulations have been performed over several years of operation to ensure that steady state conditions are achieved.

**Process stoichiometry and kinetics**

Since the wastewater is assumed to contain no carbon, and also for reasons of simplicity, heterotrophic growth has not been explicitly considered in the model. The model stoichiometry and kinetics are based on those of Hao et al. [4] and Koch et al. [12] and are summarized in Tables 1 and 2. Inhibition of $X_{AOB}$ and $X_{NOB}$ by NH$_3$ and HNO$_2$, and also inhibition of $X_{AN}$ at high nitrite concentrations, have not been considered in the model, as in the Hao and the Koch studies. In addition to endogenous respiration on oxygen and nitrate ($S_{NO_3}$), endogenous respiration on nitrite ($S_{NO_2}$) has also been included. Table 3 lists the stoichiometric and kinetic parameters.

**Results and discussion**

**Influence of bulk oxygen concentration on reactor performance**

In the autotrophic nitrogen removal processes considered, the different biomass species are competing for space in the granule and the substrate. $X_{AOB}$ (ammonium oxidizers) and $X_{NOB}$ (nitrite oxidizers) compete for oxygen, $X_{AOB}$ and $X_{AN}$ (anammox bacteria) for ammonium and $X_{NOB}$ with $X_{AN}$ for nitrite. Since nitrite is produced by $X_{AOB}$, its presence is required for the survival of the two other species. Whether $X_{NOB}$, $X_{AN}$, or both simultaneously, will be present, depends on the oxygen concentration in the bulk liquid. Ammonium and nitrite oxidizers each need oxygen, but ammonium oxidizers have a higher oxygen affinity ($K_{O_2}^{AOB} < K_{O_2}^{NOB}$, see Table 3 and [13]). On the
Table 1. Stoichiometric matrix.

<table>
<thead>
<tr>
<th>j component</th>
<th>S_{NH} [gN m^{-3}]</th>
<th>S_{NO2} [gN m^{-3}]</th>
<th>S_{NO3} [gN m^{-3}]</th>
<th>S_{O2} [gO2 m^{-3}]</th>
<th>S_{N2} [gN m^{-3}]</th>
<th>X_{AOB} [gCOD m^{-3}]</th>
<th>X_{NOB} [gCOD m^{-3}]</th>
<th>X_{AN} [gCOD m^{-3}]</th>
<th>X_{I} [gCOD m^{-3}]</th>
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<td>Ammonium oxidation</td>
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<td>-4.57</td>
<td>-1</td>
<td>-1.71</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>gN/unit comp</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>i_{NXB}</td>
<td>i_{NXB}</td>
<td>i_{NXB}^{AN}</td>
<td>i_{NXI}</td>
</tr>
</tbody>
</table>
other hand, anammox bacteria are inhibited by oxygen and require strictly anoxic conditions for growth. The biomass distribution in the granule, and the associated reactor performance, are the collective result of these interactions and characteristics. Completely autotrophic anammox is achieved only when nitrite oxidizers are completely outcompeted by the anammox bacteria.

Figure 1 shows the evolution of the bulk liquid concentration of nitrogen components over time, as well as the corresponding steady state biomass distribution profiles in the granule, for various bulk liquid oxygen levels \( (S_{O2}) \). Figure 2 summarizes the steady state reactor behaviour for a wide range of bulk oxygen concentration values. After initial nitrite \( (S_{NO2}) \) accumulation, nitrite is converted by \( X_{AN} \) to gaseous nitrogen \( (S_{N2}) \) and/or by \( X_{NOB} \) to nitrate \( (S_{NO3}) \). The competition between anammox bacteria and nitrite oxidizers results in nitrite conversion mainly to gaseous nitrogen at low bulk oxygen concentrations, whereas nitrate formation prevails at high bulk oxygen concentrations.

Similar qualitative conclusions have been reached using one-dimensional \([4]\) and two- and three-dimensional \([14]\) modelling studies of biofilms developing on a planar substratum, but the precise oxygen values at which one reaction prevails over another differ due to different biomass properties (kinetic parameters), apart from reactor operating conditions and biofilm geometry (granule size), influencing biomass loading rate and ammonium surface load. At very low oxygen concentrations \( (S_{O2} = 0.1 \text{ g m}^{-3}) \), nitrite oxidizers are completely outcompeted by anammox bacteria. However, part of the ammonium remains unconverted due to the oxygen limitation of ammonium oxidizers.
Overall, there is an optimal range of bulk oxygen concentrations, $S_{O_2}$, for which completely autotrophic ammonium removal is achieved, as reflected by the $S_{N_2}$ peak in Figure 2. Note that, for an $S_{O_2}$ of about 0.3 g m$^{-3}$ a small amount of nitrite remains unconverted, but in all other cases the bulk liquid does not contain nitrite. Initial nitrite accumulation before conversion to $S_{N_2}$ or $S_{NO_3}$ (Figure 1) is caused by the fact that ammonium oxidizers accumulate more rapidly in the reactor than anammox bacteria or nitrite oxidizers. The period of nitrite accumulation is the most extended (note the different scale for $S_{O_2} = 2$ g m$^{-3}$) for

### Table 3. Stoichiometric and kinetic parameter values.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{AOB}$</td>
<td>0.20</td>
<td>g COD g$^{-1}$ N</td>
<td>[13]$^1$</td>
</tr>
<tr>
<td>$Y_{NOB}$</td>
<td>0.057</td>
<td>g COD g$^{-1}$ N</td>
<td>[13]$^1$</td>
</tr>
<tr>
<td>$Y_{AN}$</td>
<td>0.17</td>
<td>g COD g$^{-1}$ N</td>
<td>[16]$^2$</td>
</tr>
<tr>
<td>$i_{NXB}$</td>
<td>0.083</td>
<td>g N g$^{-1}$ COD</td>
<td>For assumed composition CH$<em>{1.8}$O$</em>{0.5}$N$_{0.2}$</td>
</tr>
<tr>
<td>$i_{AN}$</td>
<td>0.058</td>
<td>g N g$^{-1}$ COD</td>
<td>For composition CH$<em>{2}$O$</em>{0.5}$N$_{0.15}$ (see [11])</td>
</tr>
<tr>
<td>$i_{NXX}$</td>
<td>0.66</td>
<td>g N g$^{-1}$ COD</td>
<td>[11] (ASM1)</td>
</tr>
<tr>
<td>$f_{XI}$</td>
<td>0.20</td>
<td>g COD g$^{-1}$ COD</td>
<td>[11] (ASM3)</td>
</tr>
</tbody>
</table>

### Kinetic parameters (at 30°C and pH 7)

- $\mu_{AOB}^{\max}$: 1.36 d$^{-1}$
- $\mu_{NOB}^{\max}$: 0.79 d$^{-1}$
- $\mu_{AN}^{\max}$: 0.052 d$^{-1}$
- $K_{NH}$: 1.1 g N$^{-3}$
- $K_{NO_2}$: 0.51 g N m$^{-3}$
- $K_{NH}$: 0.03 g N m$^{-3}$
- $K_{NO_2}$: 0.005 g N m$^{-3}$
- $K_{O_2}$: 0.3 g O$_2$m$^{-3}$
- $K_{O_2}$: 1.1 g O$_2$m$^{-3}$
- $K_{O2}$: 0.01 g O$_2$m$^{-3}$
- $b_{AOB}$: 0.065 d$^{-1}$
- $b_{NOB}$: 0.040 d$^{-1}$
- $b_{AN}$: 0.0026 d$^{-1}$
- $K_{NO3}$: 1 g N m$^{-3}$
- $K_{NO2}$: 1 g N m$^{-3}$
- $H$: 0.5

1. After unit conversion, using a typical biomass composition of CH$_{1.8}$O$_{0.5}$N$_{0.2}$, corresponding to 1.3659 g COD g$^{-1}$.
2. After unit conversion, using an anammox biomass composition of CH$_{2}$O$_{0.5}$N$_{0.15}$ (see [16]), corresponding to 36.4 g COD mole$^{-1}$ or 1.51 g COD g$^{-1}$.
3. Conversion of values given in [17] at 35°C and in [16] at 32.5°C to 30°C using relationship (written for AOB, analogous for NOB and AN)

$$
\mu_{max}^{AOB}(T) = \mu_{max}^{AOB}(T_{ref}) \exp \left( \frac{E_{a}^{AOB}(T-T_{ref})}{R \cdot T \cdot T_{ref}} \right)
$$

with $E_{a}^{AOB}$=68 kJ mol$^{-1}$ K$^{-1}$; $E_{a}^{NOB}$=44 kJ mol$^{-1}$ K$^{-1}$; $E_{a}^{AN}$=70 kJ mol$^{-1}$ K$^{-1}$ (see [15]); $R = 8.31$ J mol$^{-1}$ K$^{-1}$.
4. Calculated value at $T = 30°C$ and pH = 7 from $K_{NH}^{NOB} = 0.028$ g NH$_3$ N m$^{-3}$ and from $K_{NO2}^{NOB} = 3.2e-5$ g HNO$_2$ N m$^{-3}$ considering the T and pH dependency of the chemical equilibria $NH_4^+ \leftrightarrow NH_3 + H^+$ and $HNO_2 \leftrightarrow NO_2^- + H^+$.

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Figure 1. Evolution of bulk concentrations of ammonium ($S_{NH}$), nitrite ($S_{NO2}$), nitrate ($S_{NO3}$) and nitrogen gas ($S_{N2}$) (left: a,c,e,g). Steady state distribution of biomass ($X_{AOB}$: ammonium oxidizers; $X_{NOB}$: nitrite oxidizers; $X_{AN}$: anammox bacteria) and particulate inerts ($X_i$) in the granule, $z$ is the distance from the granule centre (right: b,d,f,h). Simulation results for various bulk oxygen levels $S_{O2}$ at fixed $r_{gran} = 0.75$ mm; $V_R = 400$ m$^3$; $S_{NH,in} = 300$ g N.m$^{-3}$. 
In order to assess the influence of the granule size on reactor performance, and more specifically on the bulk oxygen concentration range corresponding to completely autotrophic nitrogen removal (outcompetition of nitrite oxidizers by anammox bacteria), simulations have been carried out for different values of the granule radius. In these simulations, the total volume of granules in the reactor at steady state has been set constant at 100 m³, meaning that the number of granules in the reactor \(n_{\text{gran}}\) depends on the chosen granule radius: for \(r_{\text{gran}} = 0.75, 1, 1.5\) mm, \(n_{\text{gran}}\) is calculated to be \(5.66 \times 10^{10}, 2.39 \times 10^{10}\) and \(7.07 \times 10^{9}\), respectively. As the surface:volume ratio of spherical particles decreases with increasing granule radius, the associated ammonium surface load increases correspondingly, amounting to 1.875, 2.5 and 3.750 g N day\(^{-1}\) m\(^{-2}\) for \(r_{\text{gran}} = 0.75, 1, 1.5\) mm, respectively.

Figure 3 summarizes the results in terms of the steady state bulk concentrations of the nitrogen components. The corresponding fractions of biomass and particulate inert matter are given in Figure 4. As the particle size increases, it takes higher bulk oxygen concentrations to achieve complete ammonium conversion and associated nitrite formation, as well as subsequent transformation to nitrate or gaseous nitrogen.

The fraction of nitrite oxidant in the granules decreases with increasing granule size, indicating relatively more anammox conversion. This can be attributed to the increased ammonium surface load associated with larger granules, resulting in a smaller oxygen penetration depth and hence in relatively less aerobic, and more anoxic, volume – despite the fact that the outside layer with a given thickness represents a larger volume for larger particles. The relative increase in anoxic versus aerobic activity with increasing granule diameter corresponds to the results of de Kreuk et al. [5] for simultaneous N-removal (through conventional nitrification–denitrification) and P-removal, and of Ni et al. [6] for the stand-alone anammox process. Experimental confirmation for autotrophic nitrogen removal is described by Vlaeminck et al. [7].

Moreover, the bulk oxygen concentration range for anammox conversion of nitrite, as reflected in the production of gaseous nitrogen, becomes broader for larger particles. This results from the increased ammonium surface loading rate for larger granules, resulting
in relatively less aerobic volume, combined with the fact that nitrite oxidizers are more sensitive to oxygen limitation than are ammonium oxidizers ($K_{O_2}^{NOB} > K_{O_2}^{AOB}$). This implies that controlling a granular reactor becomes easier with increasing particle size, as it becomes more robust towards disturbances in bulk oxygen concentration, or to the particle size itself: assuming that the latter can be controlled by selecting particles of the desired radius.

Note also that the bulk concentration range at which steady state nitrite formation is reached increases with increasing particle size, as does the corresponding nitrite level. The bulk oxygen concentration ranges for gaseous nitrogen and nitrite formation show some overlap, which is relatively smaller for larger particles but nevertheless corresponds to higher nitrite concentrations. This means that nitrite will be present in the reactor effluent when aiming at optimal anammox conversion.
High nitrite concentrations are expected to inhibit anammox conversion. Complete inhibition of the anammox process has been reported for nitrite concentrations above 100 g N m\(^{-3}\) (see [15]), but this also depends on biomass adaptation. Since this type of inhibiting effect has not been considered in the present study due to a lack of well-documented inhibition coefficients, caution must be exercised in interpreting the simulation results relating to high nitrite concentrations and simultaneous anammox conversion.

**Effect of influent ammonium concentration**

The effect of the influent ammonium concentration on the optimal bulk oxygen concentration range for completely autotrophic nitrogen removal has been assessed in relation to particle size. The results are given in Figures 3 and 4, above. The effect of particle size on the optimal bulk oxygen concentration level appears to be more pronounced at higher influent ammonium concentrations, resulting in an even broader bulk oxygen concentration range and in anammox conversion. This is related to the more pronounced differences in ammonium surface load between different particle sizes at higher influent ammonium concentrations, in turn causing larger differences in aerobic over anoxic volume ratio in the granule.

**Conclusions**

The effect of granule size on autotrophic nitrogen removal in a granular sludge reactor has been assessed using numerical simulations. Completely autotrophic nitrogen removal by the granular sludge can be achieved through nitrite oxidizers being outcompeted by anammox bacteria. This is realized by controlling the oxygen concentration in the bulk liquid within a prescribed range. This optimal range of bulk oxygen concentration is broader for larger particles and for increasing influent ammonium concentrations. At the same time, the bulk oxygen range giving rise to nitrite formation becomes broader and partly overlaps the optimal range for anammox activity. The quantity of nitrite formed increases for larger particles and with increasing influent ammonium concentration. Operating the reactor with small granules may therefore be preferable in order that the reactor effluent should not contain nitrite. On the other hand, if the reactor effluent is further polished, selection for larger granules is advisable to facilitate oxygen control. This is typically the case for wastewater streams containing such high ammonium concentrations that the amount of nitrate associated with the anammox conversion, even though relatively small, is too high to comply with effluent standards. An example of the latter would be water streams which normally have to be recycled to the main wastewater treatment plant.

**Acknowledgements**

Eveline Volcke is a postdoctoral research fellow of the Research Foundation, Flanders, Belgium (FWO).

**References**


