Integration of methane removal in aerobic anammox-based granular sludge reactors

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ABSTRACT
Combined partial nitritation-anoxic ammonium oxidation (anammox) processes have been widely applied for nitrogen removal from anaerobic digestion reject water. However, such streams also contain dissolved methane that can escape to the atmosphere, hence contributing to global warming. This study investigates the possibility of integrating methane removal in aerobic anammox-based granular sludge reactors, through modelling and simulation. Methane removal could be established through aerobic methane-oxidizing bacteria (MOB), denitrifying anaerobic methane-oxidizing bacteria (damoB, $\text{NO}_2^- + \text{CH}_4 \rightarrow \text{N}_2 + \text{CO}_2$), and/or archaea (damoA, $\text{NO}_3^- + \text{CH}_4 \rightarrow \text{NO}_2^- + \text{CO}_2$). The simulation results demonstrated that the combined removal of nitrogen and methane was feasible at low dissolved oxygen conditions. Aerobic MOB were the main responsible microorganisms for removing methane. A sensitivity analysis of key kinetic parameters showed a shift in the methanotrophic populations depending on the most favourable parameters for each microbial group, while keeping high nitrogen and methane removal efficiencies. Possible methane stripping during aeration could be limited by increasing the depth within the reactor column at which aeration was supplied. Overall, the integration of methane removal in aerobic anammox-based granular sludge reactors seems to be a promising process option to reduce the carbon footprint from wastewater treatment.

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1. Introduction
Wastewater treatment negatively contributes to global warming by emitting the greenhouse gases carbon dioxide ($\text{CO}_2$), nitrous oxide ($\text{N}_2\text{O}$) [1], and methane ($\text{CH}_4$) [2]. $\text{CH}_4$ is a strong greenhouse gas that accounts for 34 $\text{CO}_2$ equivalents over a 100-year horizon [3]. $\text{CH}_4$ can be emitted after its formation in the sewage system or can be stripped to the atmosphere from the reject water from the anaerobic digester. Even though the energy recovery of dissolved $\text{CH}_4$ from reject water may not be economically attractive, its removal could significantly decrease the carbon footprint of wastewater treatment plants (WWTPs) [2].

About a decade ago, it was found that anaerobic $\text{CH}_4$ oxidation coupled to denitrification can take place [4]. Denitrifying anaerobic methane oxidation (damo) can
be carried out by denitrifying anaerobic methane-oxidizing bacteria (damoB), namely Candidatus Methylomirabilis oxyfera, which couple CH4 oxidation to nitrite reduction [5,6] (Equation (1)) and by denitrifying anaerobic methanotrophic archaea (damoA), such as Candidatus Methanoperedens nitroreducens, that uses nitrate as an electron acceptor [7] (Equation (2)).

\[
\text{damoB} \quad 3\text{CH}_4 + 8\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O}, \quad (1)
\]

\[
\text{damoA} \quad 2\text{CH}_4 + 8\text{NO}_3^- \rightarrow 2\text{CO}_2 + 8\text{N}_2O_2 + 4\text{H}_2\text{O}. \quad (2)
\]

Apart from its dissolved CH4 content, reject water is in the first place characterized by high nitrogen concentrations in the form of ammonium and by high temperatures, and is therefore suitable for the application of anaerobic ammonium oxidation (anammox) technology [8], implying important energy and chemical savings compared to conventional nitrogen removal over nitrate. Ammonium can be conveniently removed from reject water with a combined partial nitritation–anammox process [9,10], which can be implemented in one or two stages. Partial nitritation–anammox reactors have become widely applied at full-scale for the treatment of high-strength ammonium wastewaters [11]. A lot of research interest currently goes to their integration for mainstream treatment, following anaerobic psychrophilic treatment or an aerobic stage operated at a low sludge retention time [12,13].

Simultaneous ammonium and CH4 removal by anammox and damo microorganisms under anoxic conditions has been demonstrated feasible in lab-scale systems [14–16] and modelling approaches [17,18]. In practice, this system needs a preceding step realizing nitritation (i.e. oxidation of ammonium to nitrite by ammonium-oxidizing bacteria [AOB]) in order to achieve the complete removal of ammonium (by anammox) and of CH4 (by damoB, Equation (1)).

Simultaneous ammonium and CH4 removal in aerobic reactors, by combining partial nitritation–anammox systems with damo conversions in a one-stage system, so far has not been realized experimentally. Mathematical models are excellent tools to explore the feasibility of this new process, to give guidelines for directed experimental work and hence save time. Chen et al. [19] modelled simultaneous ammonium and CH4 removal in a membrane biofilm reactor to supply oxygen while avoiding CH4 stripping. They assumed that aerobic methane oxidizers (MOB) would not be present in the system since oxygen and CH4 diffused from opposite sides in the biofilm. Given that membrane systems are relatively costly and prone to fouling, the present study assessed the potential integration of CH4 conversion in aerated partial nitritation–anammox granular sludge reactors, reducing CH4 stripping by keeping the aeration minimal. In granular sludge reactors, biomass is grown in the form of dense, fast-settling granules, resulting in compact systems, which allow a high loading rate. Granular sludge reactors have been successfully applied for nitrogen removal through partial nitritation–anammox from reject water [11]; they also hold potential for nitrogen removal from the mainstream of WWTPs [20]. The integration of methane removal in addition to nitrogen removal in existing or future partial nitritation–anammox granular sludge reactors would extend the reactor performance with minimizing emissions of methane as a greenhouse gas, and is thus very promising. In this study, the potential of combined methane and nitrogen removal in partial nitritation granular sludge reactors is assessed for the first time.

The model presented in this study is the first to include not only damo bacteria and archaea, but also MOB as a potentially important microbial group for CH4 removal, besides the bacterial populations playing a role in nitrogen removal (AOB, nitrite oxidizing bacteria [NOB], and anammox bacteria) and heterotrophs (growing on decay products), in a one-stage aerobic anammox-based granular sludge reactor. Microbial competition in the granules was investigated through simulation, determining the key parameters that govern the presence or absence of the different populations. A sensitivity analysis was carried out to assess the influence of the microbial parameter values in this respect. Finally, the potential minimization of CH4 stripping through adequate reactor design was addressed as well.

2. Materials and methods

2.1. Modelling simultaneous nitrogen and methane removal

A model was set up to describe the growth and decay of damoB, damoA, anammox bacteria, AOB, NOB, MOB, and heterotrophic bacteria. Nitrification and anammox reactions were modelled based on Volcke et al. [21], heterotrophic transformations were modelled according to Henze et al. [22], and aerobic methane oxidation was modelled based on Arcangeli and Arvin [23]. The damoB process was modelled according to Winkler et al. [18], and the growth and decay of damoA (besides other species) were added in this study as in Chen et al. [19]. The interactions between all microbial groups involved are summarized in Table 1.

The stoichiometric matrix and kinetic expressions concerning the microorganisms involved in the CH4 and nitrogen removal (i.e. including the methanotrophic populations
Table 1. Overview of microbial competition for substrates (S) and dependencies through formed products (P) during simultaneous nitrogen and CH$_4$ removal in aerobic reactors.

| Aerobic populations | NH$_4$$^+$ | NO$_2^-$ | NO$_3^-$ | O$_2$ | N$_2$ | CH$_4$
|---------------------|----------|----------|----------|------|------|------
| AOB                 | S        | P        | S        |      |      |      
| NOB                 | S        | P        | S        |      |      |      
| MOB                 | S        | S        |          |      |      |      
| Aerobic heterotrophs|          |          |          |      |      |      
| Anaerobic populations |        |          |          |      |      |      
| Anammox bacteria    | S        | S        | P        | P    |      |      
| damoB               | S        |          | S        | P    |      |      
| damoA               | P        | S        |          |      |      |      
| Anaerobic heterotrophs (NO$_3^-$) | S | P | | | | 
| Anaerobic heterotrophs (NO$_2^-$) | P | S | | | | 

and anammox bacteria) are presented in the supplementary information (SI) in Tables S1.1 and S1.2, and the corresponding parameter values are listed in Table S1.3. Note that damoB could use both nitrite and ammonium as their nitrogen source for growth. Since nitrite is involved in the catabolic metabolism of damoB, this substrate needs to be present for the survival of the bacteria. Therefore, nitrite was considered the nitrogen source for damoB in this study, as in the model of Winkler et al. [18] and in accordance with the findings of Ettwig et al. [24]. The oxygen inhibition constants for damoB and damoA ($K_{O2,damoB/A}$) are not available from the literature and were considered to be the same as that for anammox bacteria, 0.01 g O$_2$ m$^{-3}$ [25]. The nitrite inhibition constant ($K_{NO2,damoA}$) and CH$_4$ half-saturation constant ($K_{CH4,damoA}$) for damoA were assumed to be the same as that for damoB, 40 g N m$^{-3}$ and 0.19 g COD m$^{-3}$, respectively.

2.2. Granular sludge reactor model

The abovementioned bioconversion reactions were implemented in a one-dimensional biofilm model in the Aquasim simulation environment [26]. A biofilm compartment was used, considering spherical biomass particles (granules) with a fixed granule size of 0.75 mm. The use of a fixed granule size rather than taking into account a more detailed granule size distribution is sufficient to assess the overall reactor behaviour [27], as is done in this study. The reactor volume was 400 m$^3$, of which 100 m$^3$ was occupied by particulate material, comprising both active biomass and inert generated during the decay, and the remaining volume (300 m$^3$) was occupied by the bulk liquid.

The interphase (gas–liquid) transfer rate for CH$_4$ and oxygen was included in the model to assess CH$_4$ stripping in the system (Section 3.5). The partial pressure of the dissolved gases was modelled as a function of the reactor height as in Daelman et al. [28] (see Section S6 in the SI).

2.3. Set-up of the simulation study

The reactor behaviour was simulated at a liquid flow rate of 1000 m$^3$ d$^{-1}$ and a fixed CH$_4$ influent concentration of 100 g COD m$^{-3}$ (25 g CH$_4$ m$^{-3}$) as in Winkler et al. [18] and Chen et al. [19]. The influent was assumed to not contain any other organic carbon source, which is a reasonable assumption for reject water [25] and allows straightforward interpretation of the simulation results. Ammonium was the sole source of nitrogen in the influent. Its concentration was varied over a realistic range for reject water, from 100 [29] up to 1500 g N m$^{-3}$, including typical values for reject water treated by partial nitritation–anammox [24]. A sufficiently long simulation time was applied to reach steady state in terms of both bulk liquid concentrations and solid concentration profiles within the granules.

An overview of the different simulations carried out and the specific settings for each case is provided in the SI (Table S2.1). While all bioconversions (Table S1.2) were included in most simulations, some simulations were run without damoA and/or damo B (i.e. by excluding reactions 9–10 and/or 7–8, respectively, from Table S1.2) to assess their effect on the nitrogen removal. In order to deal with the significant differences found in the literature for the half-saturation constants of the methanotrophs (methane half-saturation constant for damoA and damoB, $K_{CH4,damoB}$; ammonium half-saturation constant for MOB, $K_{NH4,MOB}$; and nitrite half-saturation constant for damoB, $K_{NO2,damoB}$), a sensitivity analysis was performed to determine their influence on the competition among methanotrophic communities (Table S2.2). Gas–liquid interphase transport was neglected in most simulations, corresponding with an ideal scenario without CH$_4$ stripping, to assess the maximum CH$_4$ conversion potential. The oxygen concentration was then set at a fixed value between 0.1 and 2.0 g O$_2$ m$^{-3}$ (with a resolution of 0.1 g O$_2$ m$^{-3}$), reflecting ideal oxygen control at a constant set point. Besides, a series of simulations were carried out to assess the extent of CH$_4$ stripping and its potential mitigation by adding the aeration at different depths in the reactor (SI, Section S6).

3. Results and discussion

3.1. Maximum nitrogen and CH$_4$ removal efficiencies

The maximum nitrogen and CH$_4$ removal efficiencies achieved as a function of the influent ammonium concentration, as well as the corresponding dissolved oxygen (DO) concentration ranges, are summarized in Figure 1.
Nitrogen removal efficiencies between 89% and 99% were obtained for influent ammonium concentrations up to 1000 g N m\(^{-3}\), when applying low optimal oxygen concentrations (<0.5 g O\(_2\) m\(^{-3}\)). At these oxygen concentrations, NOB were outcompeted by anammox bacteria and autotrophic nitrogen removal was established. For higher influent ammonium concentrations, the nitrogen removal efficiency decreased significantly, down to 52.6% for an influent ammonium concentration of 1500 g N m\(^{-3}\), because the corresponding ammonium loading rate exceeded the biomass-specific removal capacity (under-dimensioning of the reactor). The fluctuation in the nitrogen removal efficiency for influent ammonium concentrations up to 1500 g N m\(^{-3}\) (Figure 1) may be explained through its high sensitivity to the DO concentration. The nitrogen removal efficiency varied around 30.7 ± 15.7% when changing the DO by ± 0.1 g O\(_2\) m\(^{-3}\) (SI, Figure S3.2), which was the resolution for the DO concentration applied in this simulation study. The sensitivity of the nitrogen removal efficiency to the DO concentration was observed also in an aerobic anammox-based granular sludge reactor without CH4 removal [16]. Note that a broader range of optimal oxygen concentrations corresponding to maximum nitrogen removal was obtained in the presence of an organic substrate in the influent due to the conversion of nitrate to nitrite by heterotrophic communities [30].

Almost complete (>99%) CH4 removal was found for influent ammonium concentrations of 200 g N m\(^{-3}\) or higher (Figure 1). The corresponding optimal DO concentration interval giving high CH4 removal efficiencies became broader with increasing influent ammonium concentrations (Figure 1). Interestingly, this DO concentration interval always included the DO for maximum nitrogen removal. Thus, maximum CH4 removal can be achieved under the same (DO) conditions that lead to maximum nitrogen removal. Only in the case of a low influent ammonium concentration (NH\(_4\)\(^+\) = 100 g N m\(^{-3}\), keeping a constant CH4 concentration), the maximum CH4 removal was only 71% and required a slightly higher DO concentration (DO = 0.2 g O\(_2\) m\(^{-3}\)) than the one corresponding to maximum nitrogen removal (DO = 0.1 g O\(_2\) m\(^{-3}\)). The maximum CH4 removal efficiencies found in the present study (>99%), without considering CH4 stripping, are higher than those obtained in the simulation study of Chen et al. [19] (93%), where a membrane biofilm bioreactor was used and CH4 stripping was avoided by supplying oxygen through the membrane, despite the higher CH4 surface loading rate applied in this study (0.25 g COD m\(^{-2}\) d\(^{-1}\) in this study and 0.05–0.1 g COD m\(^{-2}\) d\(^{-1}\) in Chen et al. [19]). The difference in CH4 removal efficiency obtained in both studies could be related to the MOB activity, which was not included in the model of Chen et al. [19].

It can be noted that the granule size will affect the conversions, as demonstrated before for partial nitritation–anammox reactors [21,27]. For larger granules, the range of bulk oxygen concentration corresponding to autotrophic nitrogen removal (out-competition of NOB by anammox bacteria) is broader [30], while the maximum nitrogen removal capacity (N\(_2\) production) decreases and the optimal bulk oxygen concentration required to achieve this maximum nitrogen removal increases [30]. Winkler et al. [18] studied the influence of granule size on the combined conversion of methane (by damoB) and nitrogen (by anammox) in non-aerated granular sludge reactors and also found lower removal efficiencies for larger granules. Likewise, in aerated granular sludge reactors, a lower methane and nitrogen removal efficiency for larger granules due to larger diffusion limitations is expected.

3.2. Contribution of functional groups to CH4 and nitrogen removal

Considering the cases with maximum nitrogen removal (Figure 1), MOB were the main contributors to CH4 removal in most cases. Over 58% of CH4 was removed by MOB, except for influent ammonium concentrations of 100 g N m\(^{-3}\), with no MOB contribution on the CH4 removal; and 400 g N m\(^{-3}\), with only 18% CH4 removed by MOB (Figure 2). DamoB only contributed to CH4 removal for an influent ammonium concentration of 400 g N m\(^{-3}\). For influent ammonium concentrations higher than 800 g N m\(^{-3}\), the nitrogen loading exceeded

![Figure 1](image-url). Maximum nitrogen and CH4 removal efficiencies and corresponding DO concentration (range) as a function of influent ammonium concentration. Simulation results obtained for an ideal scenario, without CH4 stripping.
the biomass removal capacity, leading to high nitrite accumulation (SI, Figure S3.1), inhibiting damoA and leaving MOB as the only contributors for CH$_4$ removal. Between 400 and 800 g N m$^{-3}$, nitrite did not accumulate at the (low) DO concentrations ($\leq 0.2$ g O$_2$ m$^{-3}$) corresponding to maximum nitrogen removal efficiency, resulting in a relatively important participation of damoA in CH$_4$ removal (28–41% for influent ammonium concentrations in the range 600–800 g N m$^{-3}$).

For an influent ammonium concentration of 100 g N m$^{-3}$, damoA were the only microorganisms removing CH$_4$ (9.1% removal efficiency). DamoB and MOB did not survive in this case due to the limiting substrate availability (nitrite for damoB and oxygen and ammonium for MOB). DamoB only participated in the CH$_4$ removal for the maximum nitrogen removal scenarios for an influent ammonium concentration of 400 g NH$_4^+$ – N m$^{-3}$ (Figure 2). In this case, AOB produced more nitrite than required by anammox bacteria to convert the remaining ammonium, resulting in a slight accumulation of nitrite in the system (9.6 g N m$^{-3}$, SI, Figure S3.1C), hence creating a competitive edge for damoB (note that damoB have a lower affinity for nitrite than anammox bacteria, Table S1. 3). This conclusion is supported by a simplified calculation based on the ammonium consumed by AOB (SI, Table S5).

As for nitrogen removal, most of the nitrogen gas was produced by anammox bacteria (SI, Figure S4.1). The heterotrophs, growing on decay products, only had a small share in the direct nitrogen formation, with a maximum production of 3.4% of the total nitrogen formed for the scenario with influent ammonium of 400 g N m$^{-3}$. Note that heterotrophic bacteria could also contribute to nitrogen formation indirectly by reducing nitrate to nitrite, which could be then further converted to nitrogen gas by anammox bacteria [30]. Besides, this scenario was the only one where damoB had a relatively significant contribution (11%) to the nitrogen production, still being eight times lower than the contribution by anammox bacteria (86% of the nitrogen produced).

The damo process not only involves CH$_4$ removal, but also contributes to nitrogen removal, either directly through the conversion of nitrite to nitrogen gas by damoB, or indirectly through the conversion by damoA of nitrate to nitrite, which could further be taken up by anammox, heterotrophic bacteria, or damoB and converted to nitrogen gas. The specific contribution of the damo process to the nitrogen removal in the system was studied for those optimal scenarios (maximum nitrogen and CH$_4$ removal according to Figure 1) in which damoA and/or damoB were present. The simulations for these scenarios (influent ammonium concentrations from 100 to 800 g N m$^{-3}$, see Figure 3) were repeated without including damoA and/or damoB in the model (Figure 3). For influent ammonium concentrations of 100, 200, and 500 g N m$^{-3}$, the presence of damoA did not influence the nitrogen removal. For these scenarios, the total conversion of nitrate to nitrite (by heterotrophs and damoA) was the same whether or not the activity of damoA was included in the model (Figure S4.2), and thus the global nitrogen removal was the same -- the nitrite obtained by either damoA or heterotrophs was taken up by anammox bacteria, yielding nitrogen gas. At 300 g NH$_4^+$ – N m$^{-3}$, the presence of damoA led to a slightly higher nitrogen removal efficiency. The activity of damoA at 400 g NH$_4^+$ – N m$^{-3}$ was very low (1.9% CH$_4$ removed by damoA, Figure 2), not influencing significantly the nitrogen removal. For this specific case

Figure 2. CH$_4$ removed by each methanotrophic functional group (influent CH$_4$ concentration = 100 g COD m$^{-3}$) in terms of the influent ammonium concentration, for the scenarios with maximum nitrogen and CH$_4$ removal efficiency.

Figure 3. Total nitrogen removal efficiency at different influent ammonium concentrations obtained when considering both damoB and damoA ([ ]) only damoA ( □ ), only damoB ( Δ ), and no damo processes (○) in the model. Bulk oxygen concentration 0.1 g O$_2$ m$^{-3}$ for 100–300 g NH$_4^+$ – N m$^{-3}$ and 0.2 g O$_2$ m$^{-3}$ for 400–800 g NH$_4^+$ – N m$^{-3}$, corresponding to optimal scenarios (maximum nitrogen and CH$_4$ removal according to Figure 1).
(400 g NH$_4^+$ – N m$^{-3}$), the presence of damoB decreased the nitrogen removal in the system, from 98% (no damo processes considered) to 95% (damoB + damoA included or only damoB, since the activity of damoA was negligible) (Figure 3). At relatively high influent ammonium concentrations (600–800 g NH$_4^+$ – N m$^{-3}$), damoA were present and increased the nitrogen removal efficiency (Figure 3) through their synergetic interaction with anammox bacteria: nitrate produced by anammox bacteria was converted by damoA to nitrite, which was then again available for anammox bacteria, leading to higher nitrogen removal efficiencies. The positive effect of damoA on anammox conversion is comparable to the influence of heterotrophic bacteria, which may also improve the nitrogen formation by anammox through the conversion of nitrate to nitrite [30]. In this study, the contribution of damoA to the conversion of nitrate to nitrite is higher than that of heterotrophs, since the influent does not contain organic matter, so the growth of heterotrophs can only take place on decay products released.

### 3.3. Microbial distribution

The microbial distribution in the granular sludge system for the scenarios with maximum nitrogen removal efficiencies (Figure 1) is displayed in Figure 4 (fraction of each population) and Figure 5 (relative distribution inside the granules). AOB and anammox bacteria dominated the granules, while the fraction of methanotrophs (damoA, damoB, and/or MOB) was low (Figure 4), corresponding to the relatively high ammonium load compared to the CH$_4$ load. NOB were not present in the system at the low DO concentrations required for maximum nitrogen and CH$_4$ removal. AOB and aerobic MOB governed the aerobic outer part of the granules (Figure 5). Anammox bacteria and damoA shared the space inside the granule, and for the specific case where damoB were present (influent ammonium concentration 400 g NH$_4^+$ – N m$^{-3}$, Figure 5(A)), these bacteria were located in the inner part of the anoxic zone. The relative location of anammox bacteria and damoB is in accordance with the findings of Winkler et al. [18], for an anoxic (non-aerated) granular sludge system.

The presence of the individual methanotrophic groups (damoA, damoB, and MOB) for a range of influent ammonium and bulk oxygen concentrations is summarized in the coexistence graph (Figure 6). The regions between the thick black lines correspond to the scenarios with maximum nitrogen removal. DamoA or damoA + damoB (regions of A and A + B in Figure 6) were favoured at relatively low ammonium concentrations where the conversion of ammonium was high (see cases in Figure S3.1 at DO >0.1 g O$_2$ m$^{-3}$). In these cases, where ammonium was not in excess, the low affinity of MOB for ammonium (nitrogen source for their growth) impeded their growth and damoA and damoB took advantage over MOB, also given their higher affinity for methane ($K_{CH4,damoA}$ = 0.19 and $K_{CH4,MOB}$ = 0.26 g COD m$^{-3}$, Table S1. 3). However, when sufficient ammonium was available as nitrogen source for biomass growth, MOB were competitive because of their higher growth rate ($\mu_{max,MOB}$ = 3.5 d$^{-1}$, $\mu_{max,damoA}$ = 0.036 d$^{-1}$, and $\mu_{max,damoB}$ = 0.050 d$^{-1}$, Table S1.3). DamoA coexisted with MOB in all cases, except when there was a high accumulation of nitrite, causing their inhibition ($K_{N2O,damoA}$ = 40 g NH$_4^+$ – N m$^{-3}$). DamoA were inhibited and only MOB survived at high ammonium concentrations and DO $\neq$ 0.2 g O$_2$ m$^{-3}$ (no accumulation of nitrite at 0.2 g O$_2$ m$^{-3}$, see Figure S3.1).

Apart from the methanotrophic competition, damoB compete also with anammox bacteria for nitrite. In this respect, damoB only survived when the nitrite concentration in the system was not limiting. DamoB are the least favoured anaerobic methane oxidizing population, and never appeared alone, but always with damoA or damoA + MOB (Figure 6). The presence of damoB besides damoA and MOB typically took place when ammonium was not in excess and at relatively high DO concentrations. For an influent ammonium of 400 g N m$^{-3}$, damoB were also present at a relatively low DO of 0.2 g O$_2$ m$^{-3}$, and even were the main contributors to CH$_4$ removal (80% CH$_4$ removed by damoB, Figure 2). MOB still survived at a low DO concentration of 0.1 g O$_2$ m$^{-3}$ due to their higher or equal affinity for oxygen than the other aerobic populations ($K_{O2,MOB}$ = 0.2; $K_{O2,MOB}$ = 0.3; and $K_{O2,HA}$ = 0.2 g O$_2$ m$^{-3}$) and their high growth rate.
3.4. Sensitivity analysis for methanotrophic half-saturation constants

A sensitivity analysis was performed to assess the influence of the methane half-saturation constant for damoA and damoB ($K_{CH_4_{\text{damo}}}$), the ammonium half-saturation constant for MOB ($K_{NH_4_{\text{MOB}}}$), and the nitrite half-saturation constant for damoB ($K_{NO_2_{\text{damoB}}}$) on the presence of the methanotrophs and on the reactor performance in terms of nitrogen and CH$_4$ removal efficiencies (Table S2.2). The analysis was performed for the scenario with maximum nitrogen and CH$_4$ removal efficiencies (Figures 1 and 6). The analysis was performed for the scenario with maximum nitrogen and CH$_4$ removal efficiencies (Table S2.2). The analysis was performed for the scenario with maximum nitrogen and CH$_4$ removal efficiencies (Table S2.2). The analysis was performed for the scenario with maximum nitrogen and CH$_4$ removal efficiencies (Table S2.2).

Decreasing the methane affinity of damo species (i.e. increasing $K_{CH_4_{\text{damo}}}$) led to their out-competition by MOB, which was complete for $K_{CH_4_{\text{damo}}} \geq 1$ g COD m$^{-3}$ (Figure 7(A)). The $K_{CH_4_{\text{damo}}}$ for damoB and damoA in the present study is 30 times lower (higher affinity) than that in Chen et al. [19] ($K_{CH_4_{\text{damo}}} = 0.19$ vs. 5.888 g COD m$^{-3}$, respectively). While Chen et al. [19] did not include MOB in the model to describe their system, our results indicate that MOB could have played an important role.

Lower values of the ammonium half-saturation constant for MOB ($K_{NH_4_{\text{MOB}}}$) than the one used in this study (2 g COD m$^{-3}$) led to the dominance of MOB among methanotrophs (Figure 7(B)). Even though ammonium only serves as a nitrogen source for the biomass growth of MOB, the change in $K_{NH_4_{\text{MOB}}}$ plays an important role, especially at low ammonium concentrations, which leads to their growth limitation.

Decreasing the nitrite half-saturation constant for damoB ($K_{NO_2_{\text{damoB}}}$) (hence increasing the competitiveness for nitrite) resulted in increasing damoB in the system, but barely affected the anammox population abundance (Figure 8(A)), because of the high ammonium load compared to the CH$_4$ load (41.7 g N m$^{-3}$ h$^{-1}$ and 10.4 g COD m$^{-3}$ h$^{-1}$, respectively) and the relatively high yield of anammox bacteria (0.170 g COD [g N]$^{-1}$) compared to damoB (0.0835 g COD [g COD]$^{-1}$). The presence of MOB and damoA increased with increasing $K_{NO_2_{\text{damoB}}}$ while the overall CH$_4$ removal efficiency was not affected (Figure 8(B)). In this study, anammox bacteria are assumed to have a higher nitrite affinity.
than damoB ($K_{NO2_damoB} = 0.6$; $K_{NO2_AN} = 0.005 \text{ g N m}^{-3}$). Chen et al. [19] assumed the opposite, favouring damoB when competing with anammox bacteria for nitrite ($K_{NO2_damoB} = 0.01$; $K_{NO2_AN} = 0.05 \text{ g N m}^{-3}$). This study demonstrates that the presence of damoB is influenced by $K_{NO2_damoB}$ (Figure 8(B)). Higher values of $K_{NO2_damoB}$ as in the study of Chen et al. [19] imply a more advantageous situation for anammox bacteria relative to damoB, which could affect the CH4 removal.

### 3.5. Effect of reactor operation and design on CH4 stripping

The effect of aeration intensity ($k_{La}$) on the fate of CH4 is summarized in Figure S6. In case of surface aeration (i.e. aeration depth = 0), high nitrogen removal was achieved at the expense of high CH4 stripping (maximum nitrogen removal achieved = 98% at $k_{LaO2} = 500 \text{ d}^{-1}$ with 56.5% of CH4 stripped, Figure S6.A).

CH4 stripping could be reduced through the partial recirculation of the off-gas, as already applied in certain full-scale aerobic anammox-based granular sludge reactors as a means of aeration control [31]. Recirculation of part of the off-gas would increase the CH4 partial pressure and thus lead to less CH4 stripping. However, CH4 in the non-recirculated part of the off-gas would still end up in the atmosphere. A second option to minimize CH4 stripping, possibly combined with recirculation, would be to use subsurface aeration rather than surface aeration [28] and to locate the (CH4 containing) influent supply line inside the reactor. Installing the aeration supply deeper in the tank results in an increased gas
solubility (CH₄ and O₂), implying less CH₄ stripping, but is counteracted by a stronger saturation (in CH₄) or depletion (in O₂) of the rising gas bubbles (higher gas retention time). Figure 9 summarizes the effect of the depth of aeration supply on the amount of CH₄ stripped, consumed, and remaining unconverted in the effluent, for the cases with the highest nitrogen removal efficiency (details on the underlying simulations are given in Figure S6). CH₄ stripping was reduced from 56% to 23% (i.e. by 60%) while keeping the same nitrogen removal efficiency (98%) when increasing the aeration supply depth from 0 (i.e. in the case of surface aeration) to 8 m (Figure 9). A further increase (to 12 m) only slightly reduced the CH₄ stripping and increased the CH₄ conversion (from 77% to 80% CH₄ converted for an aeration depth of 8 and 12 m, respectively), which may not be warranted by the higher energy costs required to supply oxygen at this depth and by the slightly increased DO concentration needed to achieve the corresponding optimal nitrogen removal efficiency.

4. Conclusions

The possible integration of CH₄ removal in an aerobic anammox-based granular sludge reactor was assessed through a simulation study.

- A model was set up including not only damo bacteria and archaea, but also aerobic MOB as a potentially important microbial group for CH₄ removal, besides the bacterial populations playing a role in nitrogen removal.
- Simultaneous nitrogen and CH₄ removal was demonstrated feasible for low DO concentrations (DO <0.5 g O₂ m⁻³), achieving up to 99% removal efficiencies for both substrates in an ideal case without CH₄ stripping.
- While nitrogen removal efficiency was found to be very sensitive to the DO concentration, maximum CH₄ removal was obtained in a broader oxygen concentration interval. This DO concentration interval always included the DO for maximum nitrogen removal; thus, maximum CH₄ removal was achieved under the same DO conditions as that for maximum nitrogen removal.
- The presence of damo archaea improved the nitrogen removal efficiency by converting the nitrate formed by anammox bacteria.
• High CH$_4$ removal efficiencies were maintained at different influent ammonium and DO concentrations and when varying kinetic parameters. While a shift could be noted in the methane-oxidizing population, aerobic methane oxidizing bacteria were the main responsible microorganisms for CH$_4$ removal in all cases.

• CH$_4$ stripping during aeration could be limited by increasing the aeration supply depth within the reactor (23% CH$_4$ stripped at 8 m depth), while keeping the nitrogen removal efficiency high.

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