

Modelling simultaneous CH₄ and ammonium removal in a one-stage aerobic granular sludge reactor

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Summary of key findings

The feasibility of simultaneous ammonium and CH₄ removal in a one-stage aerobic granular sludge reactor by anaerobic ammonium oxidizing (anammox) bacteria and nitrite-dependent anaerobic methane oxidizing (N-damo) bacteria was assessed based on a one dimensional biofilm model. A simulation study was performed to assess the bacterial competition in the granules, as well as the influence of key variables such as oxygen concentration and ammonium loading rate. Limited oxygen concentration and relatively low ammonium loadings were required for coexistence of anammox and N-damo bacteria in aerobic granules. The results show that simultaneous ammonium and CH₄ removal is feasible in aerobic granules and open the door to future implementations of this technology for more sustainable reject water treatment in an one stage reactor compartment.

Background and relevance

Reject water from anaerobic digestion contains dissolved CH₄ that can potentially escape to the atmosphere during further downstream processing. CH₄ is a strong greenhouse gas (GHG) that accounts for 34 CO₂ equivalents over 100 years horizon (IPCC 2013), meaning that even small emissions of CH₄ have a substantial contribution to the carbon footprint of the wastewater treatment plants (WWTPs). The nitrite-dependent anaerobic CH₄ oxidizing (N-damo) bacteria *Candidatus Methylophilus oxyfera* (Raghoebarsing et al., 2006) have the unique capacity to simultaneously oxidize CH₄ to CO₂ and reduce nitrite to dinitrogen gas under anaerobic conditions (Ettwig et al., 2010).

Apart from its dissolved CH₄ content, reject water is characterized by high ammonium concentration and high temperatures and is therefore suitable for the application of anaerobic ammonium oxidation (anammox) technology, resulting in important energy and chemical savings compared to conventional nitrogen removal over nitrate.

The feasibility of the simultaneous CH₄ and ammonium removal by anammox and N-damo bacteria under anaerobic conditions was already studied in lab-scale systems (Luesken et al., 2011; Shi et al., 2013) and by mathematical modelling (Chen et al., 2014; Winkler et al., 2015). In practice, this system needs an additional step performing partial nitrification in order to achieve the CH₄ and ammonium removal. Ammonium can be conveniently removed from reject water with a combined partial nitrification-anammox process (van der Star et al. 2007). However, to avoid problems with CH₄ stripping in the aerated partial nitrification reactor, it is interesting to investigate the possibility of simultaneous CH₄ and ammonium removal in a one-stage system, operated with minimal aeration to avoid stripping effects. A one-stage system could be operated with granular sludge, which allows higher biomass retention time and anoxic and aerobic zones in the same unit. A one-stage configuration would have low footprint, operational and investment costs. The present study is the first to model simultaneous CH₄ and ammonium removal by anammox and N-damo bacteria in single aerobic granular sludge reactor and to assess the feasibility of such a system for reject wastewater treatment.

Results and discussion

Effect of oxygen concentration in the bulk liquid

Oxygen concentrations in the bulk liquid were varied from 0.1 to 1.5 mg O₂ L⁻¹ (Figure 1). N-damo and anammox bacteria survived in the system at low oxygen concentrations (0.2-0.3 mg O₂ L⁻¹) (Figure 1A). Higher oxygen concentrations inhibited both bacteria and led in the dominance of ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and aerobic heterotrophs (HA).

The absence of aerobic methane oxidizing bacteria (MOB) in the granules at high oxygen concentrations was explained due to their detachment from the granule and wash out of the system. Thus, the CH_4 removal efficiency at high oxygen concentrations drops sharply (Figure 1B). The optimal oxygen concentration chosen for all further simulations was $0.2 \text{ mg O}_2 \text{ L}^{-1}$ and corresponded with a nitrogen removal efficiency of 99% (Figure 1B), while did not lower the CH_4 removal efficiency.

Effect of the influent ammonium rate

The influence of ammonium loading rate on the biomass fraction in the granules is shown in Table 1. Anammox bacteria outcompeted N-damo bacteria at high ammonium loading rates, while too low ammonium loading rates did not facilitate their presence, since the formation of nitrite was limited. For the present study, ammonium loading rates of 0.75 and $1.25 \text{ kg N d}^{-1} \text{ m}^{-3}$ (corresponding with a concentration of $300 \text{ mg NH}_4^+ \text{ N L}^{-1}$ and $500 \text{ mg NH}_4^+ \text{ N L}^{-1}$) enabled the coexistence of N-damo and anammox bacteria in aerobic granules.

Biomass distribution in the granules

Figure 2 shows the biomass distribution inside of a granule. N-damo and Anammox bacteria were both located in anoxic zones of the granules. N-damo bacteria grew in the inner core due to their slower growth rate if compared to N-damo bacteria. AOB dominated the aerobic fraction.

Conclusions

- Simultaneous CH_4 and ammonium removal by N-damo and anammox bacteria in a one-stage aerobic granular sludge reactor was demonstrated feasible by simulation.
- Limited oxygen concentrations and relatively low ammonium loading rates are required for the coexistence of anammox and N-damo in aerobic granules.
- N-damo bacteria grow in inner part of the granules compared to anammox bacteria due to their slower growth rates.

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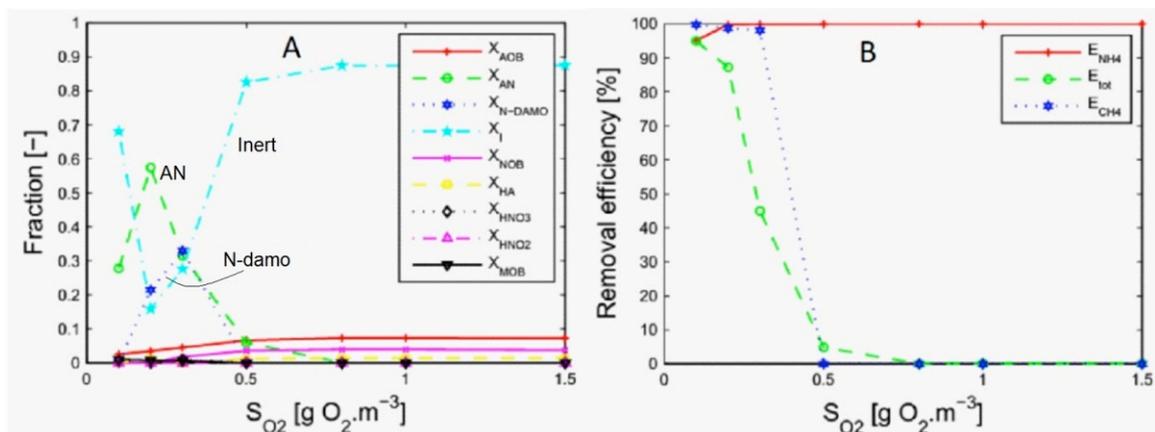


Figure 1 Influence of the bulk oxygen concentration on (A) the biomass fraction in the granules and (B) on the ammonium and CH_4 removal efficiencies. $\text{CH}_4 = 100 \text{ mg COD L}^{-1}$, granule size = 0.75 mm and influent ammonium concentration = 750 mg N L^{-1}

Table 1 Percentage of biomass in the granules at different influent ammonium loading rates. Grey rows indicate conditions favoring anammox and N-damo bacteria

Ammonium $\text{kg N d}^{-1} \text{m}^{-3}$	Percentage of biomass in the granule						
	N-damo bacteria	Anammox bacteria	AOB	NOB	MOB	Heter.	Inerts
0.25	0.0	18.0	3.0	0.0	0.3	0.3	78.5
0.75	21.5	57.0	3.5	0.0	1.0	1.0	16.0
1.25	6.0	77.0	3.5	0.0	1.0	1.5	11.0
1.88	0.0	54.0	3.5	0.0	1.0	0.5	41.0
2.50	0.0	54.0	3.5	0.0	1.0	0.5	41.0
3.75	0.0	54.0	3.5	0.0	1.0	0.5	41.0
5.00	0.0	54.0	3.5	0.0	1.0	0.5	41.0

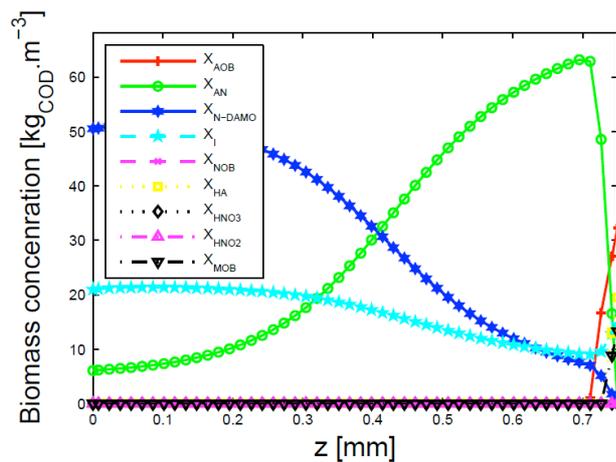


Figure 2 Distribution of the biomass in the granule (bulk oxygen concentration = $0.2 \text{ mg O}_2 \text{ L}^{-1}$, $\text{CH}_4 = 100 \text{ mg COD L}^{-1}$, granule size = 0.75 mm , influent ammonium concentration = 300 mg N L^{-1})

References

- Chen, X.M., Guo, J.H., Shi, Y., Hu, S.H., Yuan, Z.G. and Ni, B.J. (2014) Modeling of Simultaneous Anaerobic Methane and Ammonium Oxidation in a Membrane Biofilm Reactor. *Environmental Science & Technology* 48(16), 9540-9547.
- Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M.M.M., Schreiber, F., Dutilh, B.E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H.J.C.T., van Alen, T., Luesken, F., Wu, M.L., van de Pas-Schoonen, K.T., den Camp, H.J.M.O., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H., Weissenbach, J., Jetten, M.S.M. and Strous, M. (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 464, 543-548.
- IPCC, 2013. The final draft Report, dated 7 June 2013, of the Working Group I contribution to the IPCC 5th Assessment Report. In: *Climate Change 2013: the Physical Science Basis*.
- Luesken, F.A., Sanchez, J., van Alen, T.A., Sanabria, J., Op den Camp, H.J.M., Jetten, M.S.M. and Kartal, B. (2011) Simultaneous Nitrite-Dependent Anaerobic Methane and Ammonium Oxidation Processes. *Applied and Environmental Microbiology* 77(19), 6802-6807.
- Raghoebarsing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schouten, S., Damste, J.S.S., Op den Camp, H.J.M., Jetten, M.S.M. and Strous, M. (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440(7086), 918-921.
- Shi, Y., Hu, S.H., Lou, J.Q., Lu, P.L., Keller, J. and Yuan, Z.G. (2013) Nitrogen Removal from Wastewater by Coupling Anammox and Methane-Dependent Denitrification in a Membrane Biofilm Reactor. *Environmental Science & Technology* 47(20), 11577-11583.
- van der Star, W.R.L., Abma, W.R., Blommers, D., Mulder, J.W., Tokutomi, T., Strous, M., Picioreanu, C. and Van Loosdrecht, M.C.M. (2007) Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research* 41(18), 4149-4163.
- Winkler, M.K., Ettwig, K.F., Vannecke, T.P., Stultiens, K., Bogdan, A., Kartal, B. and Volcke, E.I. (2015) Modelling simultaneous anaerobic methane and ammonium removal in a granular sludge reactor. *Water Research* 73, 323-331.