Impact of organics, aeration and flocs on N_2O emissions during granular-based partial nitritation-anammox

Wan, Xinyu; Laureni, Michele; Jia, Mingsheng; Volcke, Eveline I.P.

DOI
10.1016/j.scitotenv.2021.149092

Publication date
2021

Document Version
Final published version

Published in
Science of the Total Environment

Science of the Total Environment 797 (2021) 149092

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of organics, aeration and flocs on N_2O emissions during granular-based partial nitritation-anammox

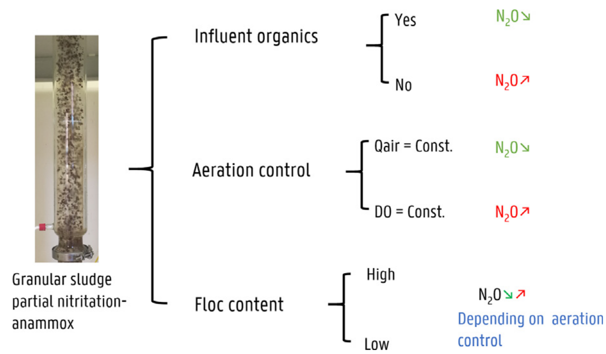
Xinyu Wan [a], Michele Laureni [a,b], Mingsheng Jia [a], Eveline I.P. Volcke [a,*]

[a] BioCo Research Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000, Ghent, Belgium
[b] Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van der Maasweg 9, Delft 2629 HZ, the Netherlands

HIGHLIGHTS

- Influent organics reduce N_2O emissions without sacrificing nitrogen removal efficiency
- Constant airflow rate control reduces N_2O emissions compared to constant DO
- Floc removal reduces N_2O under constant DO but slightly increases N_2O under constant airflow rate
- Anammox effectively decreases N_2O production by heterotrophic denitrification

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 April 2021
Received in revised form 11 July 2021
Accepted 13 July 2021
Available online 16 July 2021

Editor: Huu Hao Ngo

Keywords:
N_2O
Partial nitritation-anammox
Granular sludge
N removal
Wastewater treatment
Modelling

ABSTRACT

Partial nitration-anammox is a resource-efficient technology for nitrogen removal from wastewater. However, the advantages of this nitrogen removal technology are challenged by the emission of N_2O, a potent greenhouse gas. In this study, a granular sludge one-stage partial nitritation-anammox reactor comprising granules and flocs was run for 337 days in the presence of influent organics to investigate its effect on N removal and N_2O emissions. Besides, the effect of aeration control strategies and flocs removal was investigated as well. The interpretation of the experimental results was complemented with modelling and simulation. The presence of influent organics (1 g COD g^{-1} N) helped to suppress NOB and significantly reduced the overall N_2O emissions while having no significant effect on anammox activity. Besides, long-term monitoring of the reactor indicated that constant airflow rate control resulted in more stable effluent quality and less N_2O emissions than DO control. Still, floc removal reduced N_2O emissions at DO control but increased N_2O emissions at constant airflow rate. Furthermore, anammox bacteria could significantly reduce N_2O production during heterotrophic denitrification, likely via competition for NO with heterotrophs. Overall, this study demonstrated that the presence of influent organics together with proper aeration control strategies and floc management could significantly reduce the N_2O emissions without compromising nitrogen removal efficiency during one-stage partial nitritation-anammox processes.

1. Introduction

Partial nitritation-anammox (PNA) is a promising alternative for nitrogen (N) removal from wastewater, with considerable savings in energy, external organic carbon, sludge treatment, and associated carbon-footprint compared to conventional nitrification-denitrification

* Corresponding author at: Coupure Links 653 Building A, 9000 Gent, Belgium.
E-mail address: Eveline.Volcke@UGent.be (E.I.P. Volcke).

https://doi.org/10.1016/j.scitotenv.2021.149092
0048-9697/

(Van Dongen et al., 2001). In this process, approximately half of the ammonium (NH_4^+) is first oxidized to nitrite (NO_2^-) during nitritation by ammonium oxidizing bacteria (AOB), under aerobic conditions. In the following anaerobic ammonia oxidation (anammox) process, nitrite and ammonium are combined to form nitrogen gas (N_2). By 2014, there were more 100 full-scale applications of partial nitritation-anammox reactors, most of which were one-stage reactors, meaning that both reactions take place in a single reactor (Lackner et al., 2014).

However, as for any biological nitrogen removal process, nitrous oxide (N_2O) may be formed during the partial nitritation-anammox conversions. N_2O is a strong greenhouse gas that is 298 times stronger than CO_2 over a 100 year time horizon (IPCC, 2013). Care should be taken that N_2O emissions do not counterbalance the carbon-footprint advantages from this technology. N_2O may be produced through three biological pathways, two of which are associated with nitritation: (1) the nitrifier nitrification pathway, where N_2O is formed as a by-product during hydroxylamine (NH_2OH) oxidation; (2) nitrifier denitrification, which involves the reduction of nitrite to N_2O (Wunderlin et al., 2012). N_2O is also an obligatory intermediate during heterotrophic denitrification, which can be either an N_2O source or sink (Ali et al., 2016; Conthe et al., 2019; Wan et al., 2019). N_2O can further be produced abiotically during the nitritation process, but its contribution is negligible (<3% of total N_2O production) compared to biotic pathways (Su et al., 2019).

As only partial oxidation of ammonium to nitrite (nitritation) is required for the successful application of partial nitritation-anammox, the establishment of a balanced microbial community is essential. Granular sludge or biofilm reactors provides long sludge retention times (SRT) and make it possible to enrich the slow growing anammox bacteria (Vlaeminck et al., 2010). Oxygen-limited operation conditions are considered the most practical approach to limit NOB, as their oxygen affinity is lower than AOB (Blackburne et al., 2008; Wyffels et al., 2004). Therefore, controlling DO at a low setpoint is the most often implemented aeration strategies on partial nitritation-anammox reactors (Lackner et al., 2014). However, DO concentration alone might not always provide a good correlation with substrate depletion or biomass activity. Several simulations indicated that the optimal DO for nitrogen removal in a one-stage partial nitritation-anammox reactor decreased with smaller granules (Volcke et al., 2010;Wan et al., 2019) or more flocs (Hubaux et al., 2015) as the apparent oxygen affinities increased due to elevated oxygen penetration at smaller aggregates (Hubaux et al., 2015; Picioreanu et al., 2016; Volcke et al., 2010). As for N_2O, a previous simulation study also indicated that constant DO control failed in maintaining low N_2O emission at constant DO when flocs accumulated (Wan et al., 2021). However, these results still need to be validated with experimental data.

Selectively wasting sludge is another way to suppress NOB. This can be achieved by reducing the SRT in a suspended biomass system, as the growth rates of AOB are usually higher than those of NOB at elevated temperatures (>30 °C). For granular sludge/biofilm systems, uncoupling the SRT of flocs and granules/biofilm by selectively removing flocs successfully suppressed NOB and sustained anammox bacteria (Han et al., 2020; Laureni et al., 2019; Wett et al., 2013). This corresponds with the knowledge that anammox bacteria mainly resides in granules or aggregates while AOB/ NOB dominate in flocs (Vlaeminck et al., 2010). Yet, the effect of floc removal in this system on N_2O emissions has not yet been reported.

The presence of influent organics is another challenge in anammox-based nitrogen removal processes. Heterotrophs grow inevitably on the organics from the influent and on endogenous soluble microbial decay products (Gilbert et al., 2014; Kindaichi et al., 2004). Heterotrophic denitrifiers are can compete for nitrite with anammox bacteria at high COD/N conditions (Chen et al., 2016). However, in the presence of relatively low organic carbon concentrations, heterotrophic denitrification can co-exist with anammox, providing nitrite for anammox by reduction of residual nitrate and increasing nitrogen removal efficiency in one-stage partial nitritation-anammox reactor (Mozumder et al., 2014; Jenni et al., 2014). Heterotrophic organisms also help to suppress NOB by competing for oxygen (Li et al., 2017a, 2017b). The growth of heterotrophs in the presence of influent organics also affects N_2O emissions. Incomplete heterotrophic denitrification, caused by insufficient organic carbon (Chung and Chung, 2000; Schulthess et al., 1994), or inhibition by free nitrous acid (Zhou et al., 2008) or DO (Wunderlin et al., 2012) has been reported to cause N_2O emissions for one-stage partial nitritation-anammox reactors (Ali et al., 2016; Li et al., 2017a, 2017b). However, heterotrophic denitrification was also speculated to be a net sink of N_2O in several full-scale one-stage partial nitritation-anammox reactors (Castro-Barros et al., 2015; Domingo-Félez et al., 2014; Wan et al., 2019). It is still unclear whether heterotrophic denitrification serves as a net source or sink in one-stage partial nitration anammox reactors. The effect of influent organics on nitrogen removal and N_2O emissions definitely needs to be studied further.

In this study, a lab-scale one-stage partial nitritation-anammox reactor, with granular sludge, was operated for 337 days to study the effect of influent organics, as well as the impacts of different aeration control strategies and flocs removal on both nitrogen removal and N_2O production. Along with the long-term operation, batch tests were carried out to track the maximum activities of the functional groups and to study the N_2O formation mechanism. Besides, a mathematical model was developed and simulations were performed to provide a mechanistic interpretation of the experimental observations.

2. Materials and methods

2.1. Reactor operation

A one-stage PN/A bubble column reactor (6.5 L) was inoculated with granular sludge from a full-scale one-stage PN/A reactor in Tilburg, Netherlands. The N_2O monitoring started from day 1 (Fig. 1), after a start-up period (which lasted 17 days, reactor DO 2 g m^{-3}, influent with 500 g NH_4^+-N m^{-3} and 500 g COD m^{-3}). The reactor was continuously fed with synthetic wastewater with an influent ammonium concentration of 500-800 g N m^{-3}, and influent organic carbon present in the form of CH_3COONa, as listed in Table 1. The COD/N ratio was kept at 1, except for a period without organic carbon. The reactor was operated at an HRT of 1 day. The pH was controlled at 7.5 ± 0.1 by dosing HCl (0.1 mol L^{-1}) and NaOH (0.5 mol L^{-1}). The temperature was maintained at 38 ± 1 °C with a water jacket bath.

Different aeration control strategies were applied (Table 1): During the DO control phase (day 1-177), the airflow rate was manipulated to reach a desired oxygen concentration. This DO setpoint was adjusted manually according to the effluent nitrite concentration measurement: the DO setpoint was lowered in case two subsequent measured effluent nitrite concentration exceeded 20 g NO_2^-N m^{-3} (Fig. 1A-B). The DO sensor was cleaned regularly from day 89 on (Fig. 1E). During Q_{air} control phase (day 178-337), the reactor was operated at a constant airflow rate. The airflow rate was adjusted manually to keep the effluent nitrite and ammonium concentrations sufficiently low (Fig. 1A-B). From day 63 onwards, flocs (diameter < 0.25 mm) were removed manually from the reactor on a regular basis with a sieve, aiming to suppress the NOB (Fig. 1E). Each time, 1 L of total 6.5 L liquid in the reactor (15%) was taken and sieved for floc removal, the remaining liquid was returned to the reactor.

2.2. Batch tests

In-situ batch tests (Table 2) were conducted on 15 distinct days (indicated on Fig. 1E). Each time, the maximum activities of anammox bacteria, denitrifiers and AOB were quantified consecutively (detailed in S1.4). From day 110 onwards, maximum activities of NOB was measured simultaneously with the AOB test by adding nitrite together with ammonium.

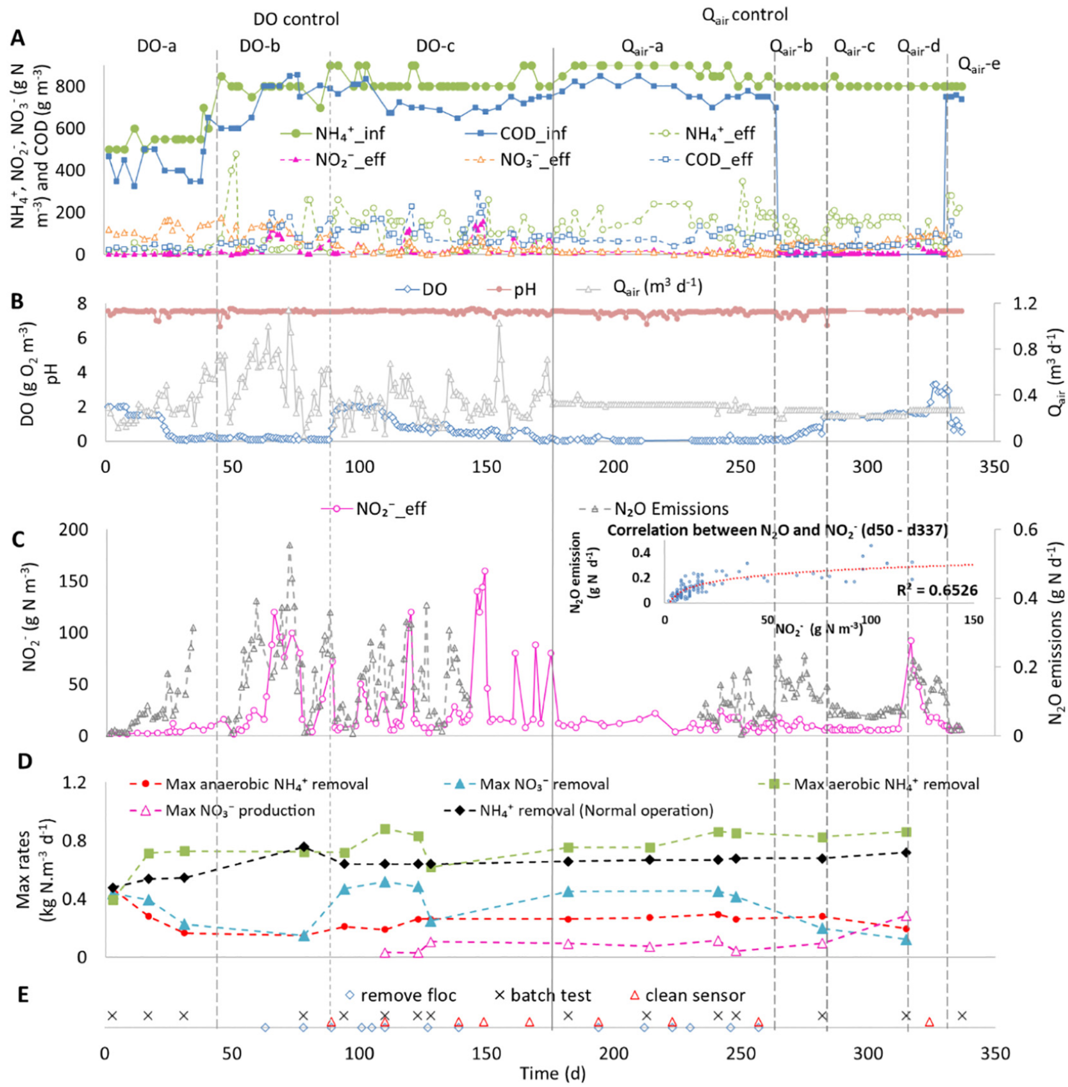


Fig. 1. Results from long-term operation of the one-stage partial nitritation-anammox reactor. (A) Influent and effluent composition; (B), reactor pH, DO and airflow rate; (C) effluent nitrite concentration, N_2O emissions and their correlation on days 50-337; D, Maximum nitrogen conversion rates in batch tests and the total NH_4^+ removal rate of the reactor at normal operation; E, Visualization of events: floc removal, sensor cleaning and batch test.

On day 337, additional batch tests were carried out to investigate the interaction between anammox and heterotrophic denitrification. Nitrate and organics were added to the reactor to stimulate denitrification. Once N_2O emissions were observed, an excess amount of ammonium was dosed into the reactor to initiate simultaneous anammox conversion. Following complete nitrate consumption and N_2O emissions decreasing to zero, organics and nitrate were dosed again to the reactor.

2.3. Reactor performance monitoring

The biomass concentration was determined as total suspended solids (TSS) and volatile suspended solids (VSS) according to standard methods (APHA, 1998). Liquid samples were filtered through 0.45 μm disposable Millipore filter. Ammonium, nitrite, nitrate and COD were determined with standard test kits (Macherey-Nagel, Germany). The

Table 1
Influent composition and aeration control strategies of the reactor.

Phase	Time	Influent NH_4^+ (g N m^{-3})	Influent organics (CH_3COONa, g COD m^{-3})	Aeration control	Description
DO-a	d1-d46	500	500	DO control	Low N load
DO-b	d47-d89	800	800		Elevated N load
DO-c	d90-d177	800	800		With sensor cleaning
Q_{air}-a	d178-d264	800	800	Q_{air}: 0.27 m^3 d^{-1}	High Q_{air} with influent organics
Q_{air}-b	d265-d280		0		High Q_{air} without influent organics
Q_{air}-c	d281-d314			Q_{air}: 0.22 m^3 d^{-1}	Low Q_{air} without influent organics
Q_{air}-d	d315-d330			Q_{air}: 0.27 m^3 d^{-1}	High Q_{air} without influent organics
Q_{air}-e	d331-d337		800		High Q_{air} with influent organics

Table 2
Batch tests conditions.

Tested process	Dosed substrates	Aeration conditions	Substrates to evaluate the activities
Anammox	NH_4^+ and NO_2^-	Bubbled with N_2	Maximum consumption rate of NH_4^+
Denitrification	CH_3COONa and NO_3^-	Bubbled with N_2	Maximum consumption rate of NO_3^-
Nitritation (and nitratation – from day 110 onwards)	NH_4^+ (and NO_2^- – from day 110 onwards)	Aerated with air	Maximum consumption rate of NH_4^+ Maximum production rate of NO_3^-
Interaction between anammox and heterotrophic denitrification	1st dosage: CH_3COONa and NO_3^- 2nd dosage: NH_4^+ 3rd dosage: CH_3COONa and NO_3^-	Bubbled with N_2	–

N_2O emissions in the off-gas were continuously measured online in the periods of day 1 to day 34, day 48 to day 143, and day 234 to day 337 (X-stream Gas analyser, EMERSON, Germany). The granules were sampled to monitor the evolution of morphology on day 1, 195, 269, and 317 with digital microscope camera (Olympus SZX9 equipped with Olympus DP21 camera, detailed in section S1.5). Granule size distributions were determined with biological-image analysis (detailed in Section S1.5) on day 1 and day 269 with Fiji software (Schindelin et al., 2012).

2.4. Simulation study

An existing 1D granular sludge partial nitritation-anammox model (Wan et al., 2019) was adapted to describe the design and operating conditions of the reactor set-up under study. The N_2O formation through nitritation was modelled according to a two-pathway model (Pocquet et al., 2016), including both nitrifier nitrification and nitrifier denitrification pathway. The heterotrophic denitrification was modelled in four steps with N_2O as an intermediate (Hiatt and Grady, 2008). The details of the bioconversions, reactor configuration and mass transfer processes described in the model were summarized in supplementary information (Sections S1.1 to S1.3). During operation at constant DO, the air flow rate was manipulated to reach the DO set point. Details on the gas-liquid transfer model are summarized in supplementary information (S1.3.1). The DO setpoint was maintained at 0.5 g O_2 m^{-3} (optimal DO setpoint for N removal). It can be noted that the setpoint in the simulation was not the same as in the experiment (between 0 and 2.5 g O_2 m^{-3} at different phase). Still, it must be noted that the DO measured by the sensor may not have been representative for the whole reactor (see further). Moreover, the model was not calibrated, since the primary goal was not to quantitatively predict the N_2O emissions, but to qualitatively understand the impacts of the operating conditions (flocs, organics, aeration) on the competition of each organism and the N_2O formation mechanisms. For the simulation of constant airflow rate control, the airflow rate was set at 0.98 m^3 d^{-1}, corresponding to the airflow rate at optimal DO.

The initial conditions for all scenarios were obtained by steady-state simulation over 5000 days. This was followed by dynamic simulations for the different dynamic scenarios, corresponding with different operations or batch tests from experiments (Table 3), in order to validate and interpret the experimental results. The different floc fractions were modelled by implementing a bifurcation from the effluent to recycle part of the detached sludge into the inlet, as described by Hubaux et al. (2015) (detailed in supplementary information S1.6). The amount of the flocs retained in the reactor was controlled by adjusting the percentage of the recycled sludge. The model was set up and run in the Aquasim 2.1d software (Reichert et al., 1996).

3. Results

3.1. Long-term reactor performance

The one-stage partial nitritation-anammox reactor was operated for 337 days, under different influent conditions and aeration strategies. The reactor was first operated with DO control (phase *DO*: day 1-177). Nitrate concentrations above 100 g N m^{-3} (more than 20% of the influent ammonium) were observed during the reactor start-up (Fig. 1A). In order to remedy this, the DO setpoint was gradually reduced from 2 to 0.1 g O_2 m^{-3} (Fig. 1B). Besides, the influent ammonium concentration was increased from 500 g N m^{-3} (phase *DO-a*) to 800 g N m^{-3} (phase *DO-b*). Since nitrate formation persisted (above 100 g N m^{-3}), part of the flocs were removed regularly (from day 60 onwards, Fig. 1E). Regular cleaning of the oxygen sensor combined with reducing the DO setpoint (Fig. 1E) finally resulted in keeping the nitrate concentration as low as 30.3 ± 21 g N m^{-3} (phase *DO-c*). Nevertheless, still some uncontrollable nitrite peaks occurred (e.g., at day 89, 109, 119, 147, 161 and 175), leading to a high average nitrite concentration (35.9 ± 42.8 g N m^{-3}). During the DO control phase, the nitrogen removal efficiency amounted to 75.5 ± 10.8% of N load (Fig. 2A), the N_2O emissions being 4.1 ± 2.8% of N removed.

The air flow rate was kept constant from day 178 onwards (phase Q_{air}). This resulted in a more stable effluent quality, with much lower nitrite (12.0 ± 12.3 g N m^{-3}) and nitrate concentrations (33.8 ± 27.5 g N m^{-3}) (Fig. 1A). As a result, the nitrogen removal efficiency also showed less fluctuation (76 ± 6.6% of N load, Fig. 2A). In addition, the N_2O emission was much lower and stable (2.4 ± 2.8% of nitrogen

Table 3
Simulation set-up. (*) Value corresponding with maximum nitrogen removal efficiency at initial conditions, namely 90%.

Scenarios	Removed flocs (% of initial flocs)	Influent NH_4^+ (g N m^{-3})	Influent COD (g COD m^{-3})	Aeration strategy	Pulse dosage
Pre-simulation (5000 days) to set initial conditions for all scenarios	–	800	800	Constant DO (*) (= 0.5 g O_2 m^{-3})	–
Stop influent COD at constant Q_{air} (cf. day 264)	–	800	From 800 to 0	Constant Q_{air} (*) (= 0.98 m^3 d^{-1})	–
Floc removal at constant DO (cf. Phase *DO*)	15%	800	800	Constant DO (*) (= 0.5 g O_2 m^{-3})	–
Floc removal at constant Q_{air} (cf. Phase Q_{air}-*a*)	15%	800	800	Constant Q_{air} (*) (= 0.98 m^3 d^{-1})	–
Batch test 1: Effect of additional COD dosage	–	–	–	No aeration	• At t = 0 h: add NO_3^- and COD • At t = 0.5 h: add COD
Batch test 2: interaction between anammox and denitrification	–	–	–	No aeration	• At t = 0 h: add NO_3^- and COD • At *t* = 0.5 h: add NH_4^+
Reference: denitrification	–	–	–	No aeration	At t = 0 h: add NO_3^- and COD

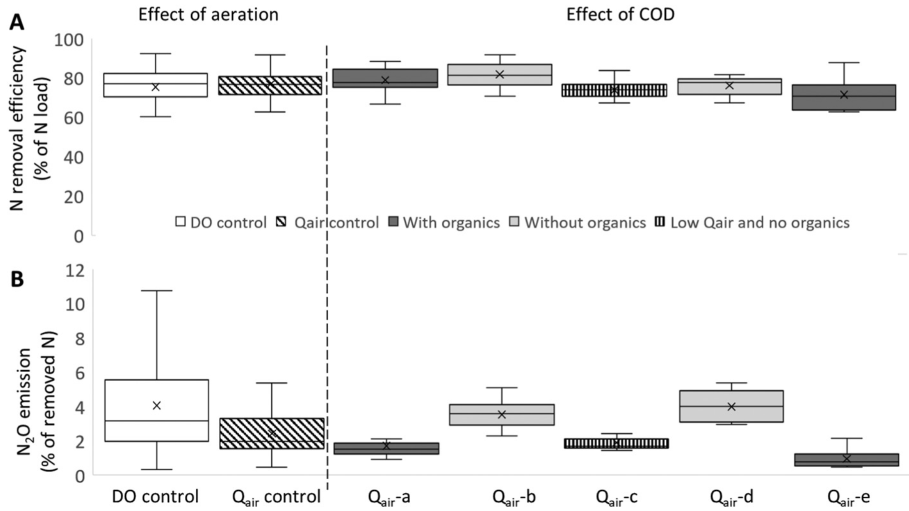


Fig. 2. Nitrogen removal efficiency (% of N load) (A) and N_2O emissions (% of nitrogen removal) (B) at different operating strategies: DO control (phase *DO*) versus airflow rate (Q_{air}) control (phase Q_{air}); Comparison between high Q_{air} with organics (phase Q_{air}-*a*, phase Q_{air}-*e*); high Q_{air} without organics (phase Q_{air}-*b* and phase Q_{air}-*d*) and low Q_{air} without organics (phase Q_{air}-*c*); The box plots indicate the interquartile range, the crosses indicated the average, the middle lines indicated the medians.

removal, Fig. 2B) at airflow rate control compared to operation with DO control.

Within the phase with constant airflow, the reactor was operated with and without influent organic carbon, as well as at different airflow rates. N_2O emissions varied at different influent compositions but the nitrogen removal was relatively stable (Fig. 2). In phase Q_{air}-*a*, the reactor was fed with both ammonium and organics, leading to a nitrogen removal of 79.0 ± 6.1% and a N_2O emission of 1.8 ± 0.9% of nitrogen removal (values of days 234-241 and days 254–264, where N_2O were measured). The fluctuating and high N_2O emissions from day 242 to day 253 (Fig. 1C) were caused by the batch test and failures in pH control. Therefore, these days were excluded in calculation of average N_2O emissions in Fig. 2. Operating the reactor without organic carbon in the influent (phase Q_{air}-*b*, days 265-330) at the same airflow rate caused a higher DO concentration (Fig. 1B) and more ammonium oxidation, resulting in lower effluent ammonium concentrations (from 149 ± 58 g N m^{-3} in phase Q_{air}-*a* to 87 ± 50 g N m^{-3} in phase Q_{air}-*b*) and higher nitrate concentrations (from 10.5 ± 7.2 g N m^{-3} to 48.8 ± 10 g N m^{-3}, Fig. 1A). Although the nitrogen removal only slightly increased (to 81.8 ± 6.6% of N load), the N_2O emissions almost doubled (3.5 ± 0.8% of nitrogen removal) compared to operation with influent organic carbon in phase Q_{air}-*a*.

Lowering the airflow rate while still operating without influent organics (at phase Q_{air}-*c*, days 283-d314) reduced the DO concentration and the ammonium oxidation rate, leading to higher effluent ammonium concentrations (161 ± 29 g N m^{-3}). The effluent nitrate concentration (45 ± 13 g N m^{-3}) gradually increased to around 80 g N m^{-3} at the end of this period, corresponding to a nitrogen removal efficiency of 73.8 ± 4.1% of N load. The N_2O emissions were lower (1.9 ± 0.5% of nitrogen removal) than in phase Q_{air}-*b* which had a higher airflow rate but higher than in phase Q_{air}-*a* when the influent contained organics. To confirm these observations, the airflow rate was increased again at phase Q_{air}-*d* (days 317 to 330), leading to a drop in the effluent ammonium concentration and a slight increase in the effluent nitrate concentration, corresponding with a nitrogen removal efficiency of 76.1 ± 5.2%. The N_2O emissions increased to 4.0 ± 0.9% compared to operation at a lower airflow rate (phase Q_{air}-*c*). Again, adding organic carbon to the influent (phase Q_{air}-*e*, days 330-337), led to a decrease in the N_2O emissions (1.0 ± 0.6% of nitrogen removal). The nitrogen removal efficiency amounted to 71.5 ± 8.9%.

On top of the aeration and influent organics, the N_2O emission dynamics were also influenced by floc removal (Fig. 3) and pH variations (Fig. S2). During operation with DO control, floc removal resulted in a drop in the N_2O emission and a pH decrease led to a decrease in N_2O emissions (Fig. S2A). However, at fixed airflow rate, N_2O emissions were slightly higher after floc removal and a pH drop led to a short peak of N_2O emissions, which was more pronounced in the presence of influent organics (Fig. S2B) than in the case without influent organics (Fig. S2 C). The effects of pH drop were further discussed in the supplementary information (Section S2.1.2).

3.2. Biomass activity and N_2O emissions in batch tests

In-situ batch tests were carried out to track the dynamics of the maximum activity of each functional guild as proxy for their relative abundance (Fig. 1D). At the beginning (phases *DO-a* and *DO-b*), both the maximum rates of anaerobic ammonium oxidation and nitrate reduction decreased as the reactor was over-aerated at that period, which was also evidenced by the high effluent nitrate concentration at that period (Fig. 1A). From phase *DO-c* onwards, the DO sensor was cleaned regularly (Fig. 1E) so the applied airflow rate was lower (Fig. 1B). As a result, the maximum rates of nitrate reduction and anaerobic

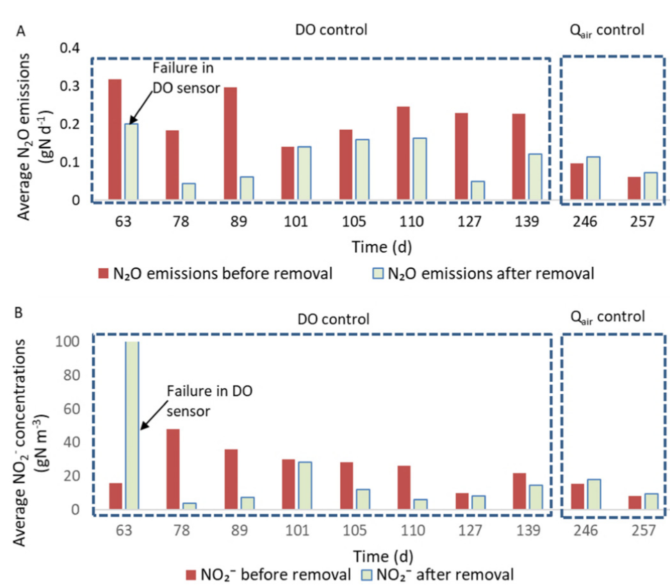


Fig. 3. Three-day average N_2O emissions (top) and nitrite concentrations (bottom) before and after floc removal.

ammonium oxidation increased at the beginning of the phase *DO-c* (Fig. 1D), corresponding to a lower effluent nitrate concentration (Fig. 1A). The absence of influent organics (in phase Q_{air}-*b*) resulted in a significant decrease in maximum nitrate reduction rate and increase in maximum nitrate production rate but did not significantly affect the maximum rates of anaerobic ammonium oxidation and aerobic ammonium oxidation. Under DO control, at day 128, a batch test was carried out immediately after floc removal, showing a significant drop in the maximum aerobic ammonium oxidation rate and nitrate reduction rate but not affecting the maximum anaerobic ammonium oxidation rate. However, at constant airflow rate (phase Q_{air}-*a*), floc removal (d246) resulted in a slight drop in maximum rates of aerobic ammonium oxidation, nitrate reduction, anaerobic ammonium oxidation and nitrate production.

3.3. N_2O during heterotrophic denitrification

During the denitrification tests, N_2O emissions showed different patterns before and after floc removal and their dynamics depended on the initial organic concentrations. In a batch test before floc removal (Fig. 4A), N_2O emissions were observed immediately after dosing organics and nitrate. However, after floc removal (Fig. 4B), a lag between dosages and N_2O emissions was observed. In the batch test with higher initial organics concentrations (Fig. 4C), the lag was extended. In the batch test after 50 days without influent organics, an extremely long lag was observed before the N_2O peak (Fig. 4E). The dosage of additional organics upon the occurrence of N_2O occurred in the batch tests of day 248 (Fig. 4D) and day 315 (Fig. 4E) stimulated N_2O emissions. In all heterotrophic denitrification batch tests, N_2O emissions were in accordance with the total nitrite reduction rate ($(C_{NO2-}(t+\Delta t) - C_{NO2-}(t)) / \Delta t - (C_{NO3-}(t+\Delta t) - C_{NO3-}(t)) / \Delta t$, Fig. 4, purple line).

In another batch test to study the interaction between anammox and heterotrophic denitrification and its effect on N_2O formation by heterotrophic denitrification, ammonium was added upon the observation of N_2O emissions (66 min after dosage of organics and nitrate, Fig. 5). Although, the N_2O emissions remained increasing, the acceleration in N_2O emissions ($d_{emissions}$/dt) dropped immediately after the addition of ammonium, indicating the reduced N_2O production rate by heterotrophic denitrification. In the following denitrification test in the presence of ammonium (from 140 min to 250 min, Fig. 5), the N_2O emissions were significantly reduced.

3.4. Simulated N_2O emissions and microbial composition dynamics

Model simulations were carried out to provide a mechanistic interpretation of the experimental observations at different scenarios, namely floc removal at different aeration control strategies, stopping influent organics feeding and batch tests.

The simulation results confirmed that floc removal led to a drop in N_2O emissions and nitrite concentrations at constant DO but stimulated N_2O emissions and nitrite accumulation at constant airflow (Fig. 6A). Before floc removal, flocs account for 14.8% of the total biomass in the reactor and 38% of AOB, 84% of heterotrophs and 3.2% of anammox were located in flocs. Removing 15% of flocs wasted 2.8% of total biomass, including 8.2% of AOB, 12% of heterotrophs and 0.9% of anammox from the reactor (Fig. S4). This indicated that floc removal mainly reduced the presence of AOB and heterotrophs as they are more prevalent in flocs than anammox bacteria (Vlaeminck et al., 2010).

Stopping influent organics feeding at constant airflow rate led to higher DO concentration, as well as increased nitrite formation and N_2O emissions (Fig. 6B). The absence of influent organics also increased the fractions of AOB, NOB and anammox (Fig. 53A). On the contrary, the fraction of heterotrophs declined, leading to lower N_2O consumption rate (Fig. 53B). The N_2O production rate by AOB showed a peak immediately after the absence of influent organics and decreased gradually due to the competition for nitrite by NOB.

During the denitrification batch test, an N_2O emissions increased rapidly when organics became limiting (Fig. 6C). Replenishing organics led to clearly reduced N_2O emissions (Fig. 6E), which dropped to zero once nitrite was depleted (Fig. 6F). Adding ammonium during the denitrification immediately decreased the nitrite concentration (Fig. 6H) and N_2O emissions (Fig. 6G).

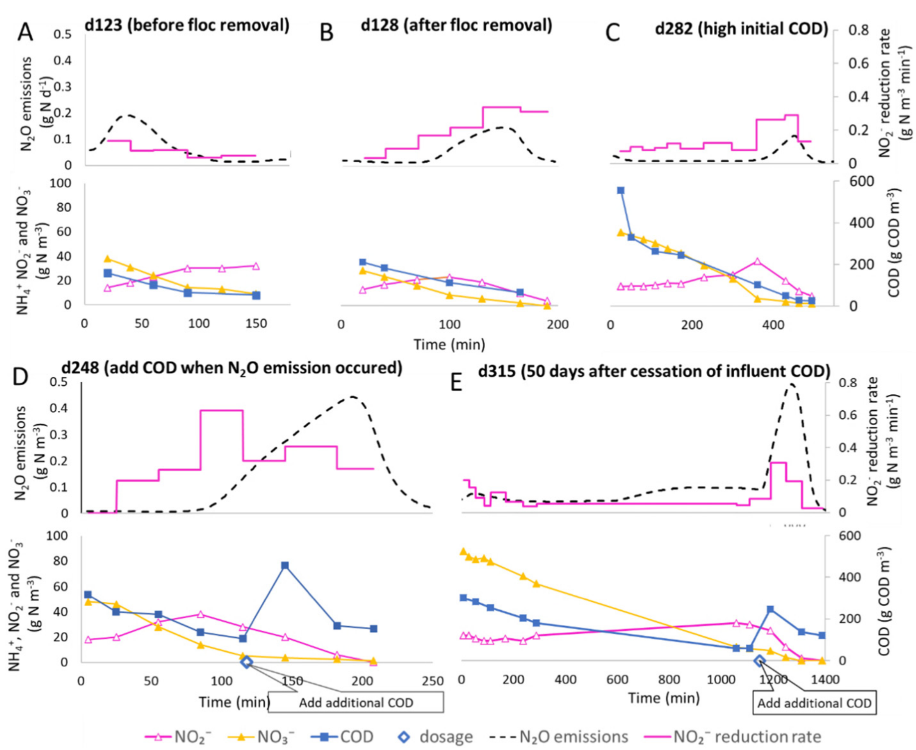


Fig. 4. Batch test results: substrate concentrations, N_2O emissions and nitrite reduction rate dynamics.

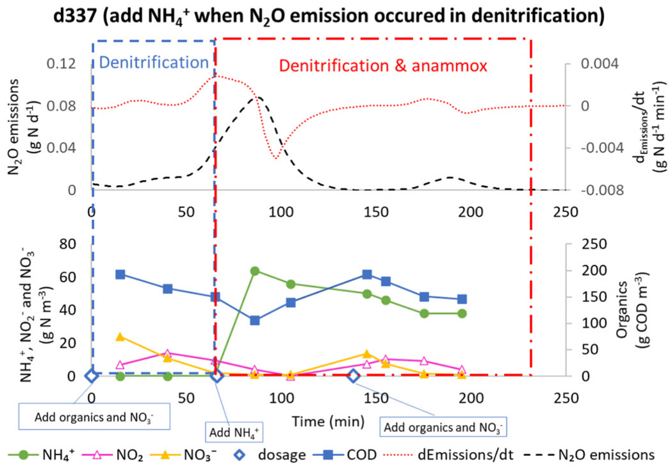


Fig. 5. N_2O emissions and substrates concentrations during denitrification when ammonium was added.

4. Discussion

4.1. Influent organics can reduce N_2O emissions without sacrificing nitrogen removal efficiency

The presence of organics did not significantly affect the overall nitrogen removal efficiencies at constant airflow rate (Fig. 2A), although the effluent ammonium and nitrate differed at different influent organics conditions. On the one hand, the presence of organics stimulated the growth of heterotrophs, competing for oxygen with AOB thus reducing the ammonium oxidation rate. This was evidenced by the higher effluent ammonium concentration in the presence of organics in phase Q_{air}-*a*. On the other hand, the presence of organics reduced the residual effluent nitrate and improved the overall N removal. The competition for oxygen by heterotrophs helped to suppress NOB in this reactor, decreasing the nitrate produced by NOB. This is confirmed by the lower maximum nitrate production rate in the presence of influent organics (Fig. 1D). In addition, the presence of influent organics stimulated the heterotrophic denitrification, consuming nitrate produced by anammox. Therefore, although the presence of influent organics led to higher effluent ammonium, the overall nitrogen removal did not decrease significantly as the effluent nitrate was diminished by stimulated denitrification and competition for O_2 on NOB.

While nitrogen removal efficiency was not affected, the N_2O emissions decreased significantly in the presence of influent organics. As indicated in Fig. 2B, N_2O emissions in all the periods with organics in the influent were lower than for the periods without influent organics.

One reason is that influent organics lead to a reduced ammonium oxidation rate, implying less nitrite accumulation and N_2O formation via nitrifier denitrification pathway. This was evidenced by both experiments (Fig. 1C, phase Q_{air}-*b*) and simulations (Figs. 6B, S5B). Besides, influent organics can also stimulate the N_2O consumption by heterotrophic denitrification by alleviating the carbon limitation to the N_2O reduction step. This is confirmed by the simulation results, where the N_2O reduction rate by heterotrophic denitrification is much higher in the presence of influent organics (Fig. S5B). This is also in line with the results of the batch tests (Fig. 4B, D, E), where no N_2O emission was observed until the COD concentration became limiting (around 150 g COD m^{-3}, note that part of the residual COD was slowly biodegradable COD accumulated from cell decay during the bath test) and the lag between the dosage and observation of N_2O emissions was extended with a higher initial COD concentration (Fig. 4D). The positive effect of influent organics on N_2O mitigation was also reported in previous simulation studies (Chen et al., 2019; Wan et al., 2019) and was experimentally confirmed in this study.

However, adding organics at high nitrite concentrations did not reduce but stimulate N_2O emissions in the batch tests (Fig. 4C and E). This can be explained by the inhibition of free nitrous acid (HNO_2) on the N_2O reduction step (Zhou et al., 2008) caused by accumulated nitrite (Fig. 4D and E). Therefore, adding organics when N_2O reduction step was inhibited only increased nitrite reduction rate (Fig. 4C and E) and temporally stimulated the N_2O accumulation. Contrary to the experimental observation, replenishment of organics in the middle of denitrification

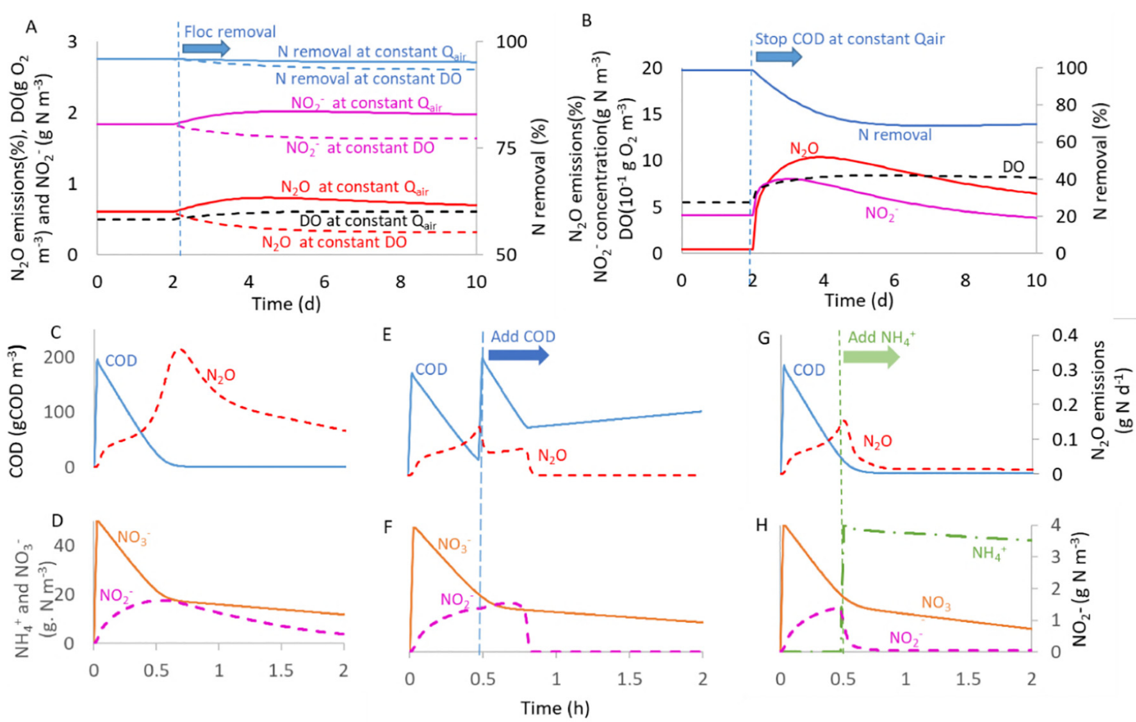


Fig. 6. Simulated N_2O emissions and substrate concentrations at different dynamic scenarios. A: N_2O emissions after floc removal; B: N_2O emissions after stopping influent organics dosing; C&D: N_2O production from heterotrophic denitrification in batch tests; E&F: Effect of dosing ammonium on N_2O production during denitrification; G&H: Effect of dosing organics on N_2O production during denitrification.

led to the drop of N_2O emissions in the simulation (Fig. 6E). This is because the inhibition of free nitrous acid was not considered in the model, and the N_2O consumption by denitrification was stimulated without free nitrous acid inhibition. In an additional simulation where the N_2O reduction step was inhibited by free nitrous acid, adding COD in the middle of denitrification stimulated N_2O production (Fig. S6). Overall, it is clear that nitrite accumulation needs to be avoided to reduce N_2O emissions. This condition is typically fulfilled in well-operated one-stage partial nitritation-anammox reactors: nitrite concentrations are typically low (less than 10 g N m^{-3} under normal operating conditions in this study) and the pH is about neutral, thus the inhibition of free nitrous acid is negligible.

High concentrations of influent organics have been reported to be inhibitory to anammox and lead to its out competition (Chen et al., 2016; Leal et al., 2016). In this study, the anammox activity and the overall nitrogen removal efficiency were relative stable during the long-term operation with an influent organics concentration of 800 g COD m^{-3} (COD/N ratio 1 g COD g N^{-1}). Successful application of partial nitritation-anammox reactor at high influent organics (COD/N ratio 1.4 g COD g N^{-1}) with an improved N removal efficiency was also reported before (Jenni et al., 2014). Likely, one-stage partial nitritation-anammox reactors allow a higher influent organics concentration than anammox reactors, as part of the influent organics were oxidized by ordinary heterotrophic organisms. Still, influent organics increased the amount of flocs and filamentary structures around the granules in this study (Fig. S1), as also reported in an anammox reactor (Pijuan et al., 2020). This could lead to a reduced SRT and more efforts on sludge retention would be required. The variations in granule morphology could further affect the N_2O emissions, as discussed in the next sections.

4.2. Airflow rate control leads to stable reactor performance and low N_2O emissions

Controlling the oxygen supply is crucial in operating a one-stage partial nitritation-anammox reactor. The oxygen supply needs to be low enough to have nitrite oxidizers outcompeted by anammox bacteria, but still high enough to enable full ammonium conversion. Keeping the DO setpoint low is considered a practical approach to not only suppress NOB due to lower oxygen affinity of NOB than AOB (Blackburne et al., 2008; Wyffels et al., 2004), but also to achieve partial nitritation by limiting the oxygen availability for AOB.

In this study, two aeration strategies were tested experimentally, namely constant DO and constant airflow rate. Controlling the bulk DO concentration at a constant setpoint, as commonly applied in practice (Lackner et al., 2014), resulted in a more fluctuating nitrogen removal efficiency and higher N_2O than when applying a constant airflow rate (Fig. 2). DO setpoint control resulted in accumulation of nitrate and nitrite even when DO was decreased to 0.1 g O_2 m^{-3} (Fig. 1A). This could partly be attributed to the growth of biofilm on the DO probe, causing a deviation in the DO measurement resulting in over-aeration. Regular cleaning of the DO sensor combined with decreasing DO setpoint successfully suppressed NOB (Fig. 1D). Still, fluctuations in nitrite and nitrate were observed, probably caused by random detachment and attachment of biofilm on the DO sensor. Therefore, frequent adjustments to the DO setpoint were required in phase *DO-c* (Fig. 1B) even if the DO sensor was cleaned periodically.

Apart from the offsets in the DO measurements, variations in floc fractions and granule sizes in the reactor could be additional reasons for the fluctuating effluent nitrite and nitrate concentrations at constant DO. A decreasing granule size or increasing floc fraction decreases the apparent oxygen affinity constant of AOB and NOB as well as the apparent inhibitory coefficients on anammox bacteria, due to the higher oxygen penetration in small aggregates (Picioreanu et al., 2016; Volcke et al., 2012). Therefore, even if DO concentration could be maintained constant in practice, the occurrence of peaks in nitrite and nitrate concentration are likely to occur with varying granule size and floc content. The effect of granule size or floc contents on the optimal DO concentration in one-stage partial nitritation-anammox reactors was also reported in several simulation studies (Hubaux et al., 2015; Mozumder et al., 2014; Wan et al., 2019).

In the case of constant airflow, variations in apparent oxygen affinities are compensated by the changes in the DO concentrations. Therefore, the effluent nitrite and nitrate concentrations show less fluctuation at constant airflow rate. The experimental results from this study thus confirm earlier simulation results indicating that constant airflow rate control leads to a better effluent quality than constant DO control under fluctuating floc amounts (Wan et al., 2021).

Constant DO control also leads to much more pronounced fluctuations in N_2O emissions than constant airflow, with a much higher average N_2O emission level for the same influent compositions. The associated fluctuations in the oxygen transfer rate may indeed lead to fluctuations in ammonium oxidation rate and related N_2O production by nitrifier nitrification. Besides, high nitrite peaks caused by fluctuations in ammonium oxidation at constant DO control can stimulate the N_2O production by nitrifier denitrification (Chandran et al., 2011). In addition, the nitrite peaks result in high free nitrous acid (HNO_2), which is inhibitory to the N_2O reduction by heterotrophic denitrification. Zhou et al. (2008) reported inhibitory concentrations of 0.0007–0.001 g HNO_2-N m^{-3} corresponding to a total nitrite concentration of 17 to 24 g NO_2^-N d^{-1} under the condition of this study (pH 7.6 and T 37 °C) – which were indeed reached here. Moreover, airflow rate fluctuations as such are known to cause increased N_2O emissions in one-stage partial nitritation-anammox reactors: Castro-Barros et al. (2015) reported N_2O emissions peaking at the transient from low aeration periods to high aeration periods during a full-scale monitoring campaign. The experimental results from this study thus confirm previous simulation results indicating that constant airflow rate leads to a better effluent quality than constant DO control (Wan et al., 2021).

In full-scale reactors, the application of a constant airflow may not be sufficient to reach stable nitrogen removal, since the influent nitrogen load may vary. Airflow rate control based on effluent nitrite and ammonium concentrations rather than constant DO control is then recommended to reduce N_2O emission and suppress NOB (Joss et al., 2011), as it can strictly control the oxygen transferred into the reactor and avoid over-aeration.

4.3. Effect of floc removal on N_2O dynamics depends on aeration control strategy

During the long-term operation, an accumulation of flocs and decreasing of granule size were observed in the reactor (Fig. S1), which was likely due to the different hydraulic conditions between the lab-scale reactor and the full-scale reactor where the granules were taken from. The relatively high amount of easily biodegradable organics in the influent, which stimulated the growth of fast growth organisms (heterotrophs), could be another reason for the smaller granule size in this reactor (De Kreuk and van Loosdrecht, 2004). Smaller granules and flocs contain a higher aerobic fraction than larger aggregates, resulting in a relatively higher abundance of AOB and heterotrophs than anammox bacteria. This is in line with the experimental results showing that anammox bacteria mainly resides in granules while AOB and NOB dominate in flocs (Vlaeminck et al., 2010). Therefore, the flocs were removed regularly from the reactor in the long-term operation to avoid NOB accumulation.

Operating at constant DO, floc removal led to a drop in N_2O (Fig. 3A). Floc removal implies a relatively higher waste of AOB than anammox from the reactor (simulation results, Fig. S4), which led to a clear drop in maximum ammonium oxidation rates, without affecting the maximum rates of anammox (batch tests, Fig. 1D d125). The decreased ammonium oxidation rate upon floc removal leads to lower effluent nitrite concentrations and N_2O emissions (Fig. 3) at DO control. Besides, removing flocs decreased the oxygen consumption and thus a somewhat lower airflow rate was required to maintain the bulk DO, decreasing the stripping and N_2O emissions slightly. These observations are in agreement with previous simulation studies indicating that more flocs led to higher N_2O emissions at constant DO (Liu et al., 2020). In contrast, when aeration airflow rate is kept constant, floc removal slightly increases the N_2O emissions (Figs. 3A and 6A). Floc removal at constant airflow rate results in a higher DO concentration (Figs. 1B and 6A) because of reduced total oxygen consumption. The elevated DO concentration stimulated the ammonium oxidation, despite the fact that some AOB were removed, and increased DO inhibition on anammox. As a result, nitrite accumulates and stimulates the N_2O emissions after floc removal at constant airflow rate. The results of this work thus provided experimental confirmation of previous simulation-only studies (Liu et al., 2020; Wan et al., 2021) that reported the presence of flocs do affect N_2O emissions.

4.4. N_2O turnover mechanisms

Consistently with previous modelling (Peng et al., 2015; Wan et al., 2019) and experimental (Blum et al., 2018a) results, the contribution of N_2O formation pathways strongly depended on the applied aeration strength. During phase *DO-a*, the high aeration strength (inferred from the low residual ammonium concentrations and high effluent nitrate concentrations) resulted in low effluent nitrite concentrations together with significant N_2O emissions likely dominated by nitrifier nitrification. High aeration results in higher ammonium oxidation rate and thus NH_2OH production, substrate for nitrifier nitrification. In turn, nitrifier denitrification is linked to the availability of nitrite (Chandran et al., 2011; Okabe et al., 2011; Wrage et al., 2001) and inhibited by high DO (Harris et al., 2015; Ma et al., 2017). From phase *DO-b* onwards, when a more limiting aeration was imposed (indicated by the lower effluent nitrate and higher residual ammonium concentrations), the N_2O emissions strongly correlated with the effluent nitrite concentration (Fig. 1C). High nitrite concentrations favour nitrifier denitrification pathway while at the same time the resulting free nitrous acid concentration likely inhibited the N_2O reduction by heterotrophic denitrifiers (Zhou et al., 2008). The correlation between nitrite and N_2O was also reported in another lab-scale reactor with intermittent aeration without influent organics (Blum et al., 2018b). Thus, limiting nitrite accumulation in one-stage partial nitritation/anammox reactors is paramount to limit N_2O emissions.

Incomplete heterotrophic denitrification has been also speculated to be, on the one hand, a N_2O source in anammox reactors primarily due to carbon limitation or inhibition of N_2O reduction by free nitrous acid (Jia et al., 2018; Pijuan et al., 2020). On the other hand, several simulation studies support the role of heterotrophic denitrification rather as net N_2O sink in one-stage partial nitritation-anammox reactors (Chen et al., 2019; Lang et al., 2019; Wan et al., 2019). While direct experimental evidence is currently lacking, net N_2O consumption has been observed in the anoxic period of intermittently aerated full (Castro-Barros et al., 2015) and lab-scale (Domingo-Félez et al., 2014) one-stage partial nitritation-anammox reactors. In our study, excess COD was present in the influent, and the relatively low effluent nitrite concentration ($<$10 g N m^{-3} under stable operation) was lower than the reported inhibitory threshold (Zhou et al., 2008). Also, in one-stage partial nitritation-anammox reactors, anammox reduced the availability of substrates for N_2O production by heterotrophic denitrifiers, probably leading to less N_2O formation than consumption (N_2O from other pathways) in heterotrophic denitrification. Therefore, heterotrophic denitrification was speculated as a net N_2O sink in this reactor. This was also supported by the decreased average emissions in presence of influent COD (Fig. 2B).

What's more, our simulations (Fig. 6G, H) and experiments (Fig. 5) suggested that anammox activity reduced N_2O formation by heterotrophic denitrification under anoxic conditions. In the simulations, anammox competed for nitrite with denitrifiers (Fig. 6H), limiting nitric oxide and subsequent N_2O production. In the experiments however, the addition of ammonium to activate anammox resulted in a reduction of N_2O accumulation during heterotrophic denitrification even at non-limiting nitrite concentrations of 10 g N m^{-3} (affinity constant of denitrifiers is 0.2 g N m^{-3}, Table S4). Owing to the recently discovered ability of anammox to grow with NO and ammonium as sole substrates (Hu et al., 2019), it is here tempting to speculate that the reduced N_2O emissions resulted from the direct competition for NO between anammox and heterotrophic denitrifiers. Unfortunately, the kinetics of anammox metabolism on NO remain as yet unknown and thus the reaction could not be included in the model. To further understand the potential positive role of anammox in controlling N_2O emissions, more experimental characterizations of anammox NO turnover are warranted.

In sum, our results indicate that heterotrophic denitrification acted as net N_2O sink during stable operation and suggest a positive role of anammox in consuming NO and thus reducing heterotrophic N_2O production.

5. Conclusions

A one-stage granular sludge partial nitritation-anammox reactor, was operated for 337 days. Model simulations were performed to aid in the mechanistic interpretation of the experimental observations.

- The presence of influent organics (COD/N = 1) significantly reduced N_2O emissions at constant airflow rate, by decreasing N_2O formation by AOB and increasing consumption by heterotrophs. The overall nitrogen removal efficiency was not affected, even though the effluent nitrate concentration was somewhat lower at the expense of a higher ammonium concentration.
- DO control failed to maintain low effluent nitrite and nitrate concentrations and led to significant fluctuations in N_2O emissions, while airflow rate control not only improved the effluent quality but also reduced N_2O emissions.
- Floc removal had contradictory effects on N_2O emissions depending on the aeration control strategy: at constant DO, removing flocs reduced ammonium oxidation rate and associated N_2O emissions; if a constant airflow was applied, floc removal elevated DO and nitrite concentrations, slightly increasing N_2O emissions.
- Nitrite accumulation should be avoided, as it stimulated the N_2O production by nitrifier denitrification and heterotrophic denitrification, and likely inhibited N_2O reduction by heterotrophic denitrification.
- Anammox bacteria potentially play a positive role in consuming NO and thus reducing heterotrophic N_2O production.

Credit author statement

Xinyu Wan: Conceptualization, Original draft, Investigation, Formal analysis, Validation, Software, Review & editing. **Michele Laureni**: Conceptualization, Review & editing, Supervision. **Mingsheng Jia**: Conceptualization, Investigation, Validation, Review & editing. **Eveline I.P. Volcke**: Conceptualization, Supervision, Review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The doctoral research work of Xinyu Wan was supported by the China Scholarship Council (CSC). Michele Laureni was supported by a VENI grant from the Dutch Research Council (NWO) (project number VI.Veni.192.252) and by a FWO postdoctoral fellowship (1223820N). Mingsheng Jia was supported by China Scholarship Council (CSC) and the special research fund (BOF) from Ghent University.

Appendix A. Supplementary information

Supplementary information to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149092.

References

Ali, M., Rathnayake, R.M.L.D., Zhang, L., Ishii, S., Kindaichi, T., Satoh, H., Toyoda, S., Yoshida, N., Okabe, S., 2016. Source identification of nitrous oxide emission pathways from a single-stage nitritation-anammox granular reactor. Water Res. 102, 147–157.

Apha, 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. American Water Works Association and Water Environmental Federation, Washington DC.

Blackburne, R., Yuan, Z., Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19, 303–312.

Blum, J., Jensen, M.M., Smets, B.F., 2018a. Nitrous oxide production in intermittently aerated partial nitritation-anammox reactor: oxic N_2O production dominates and relates with ammonia removal rate. Chem. Eng. J. 335, 458–466.

Blum, J.M., Jensen, M.M., Smets, B.F., 2018b. Nitrous oxide production in intermittently aerated partial nitritation-anammox reactor: oxic N_2O production dominates and relates with ammonia removal rate. Chem. Eng. J. 335, 458–466.

Castro-Barros, C.M., Daelman, M.R.J., Mampaey, K.E., van Loosdrecht, M.C.M., Volcke, E.I.P., 2015. Effect of aeration regime on N_2O emission from partial nitritation-anammox in a full-scale granular sludge reactor. Water Res. 68, 793–803.

Chandran, K., Stein, L.Y., Klotz, M.G., van Loosdrecht, M.C.M., 2011. Nitrous oxide production by lithotrophic ammonia-oxidizing bacteria and implications for engineered nitrogen-removal systems. Biochem. Soc. Trans. 39, 1832–1837.

Chen, C., Sun, F., Zhang, H., Wang, J., Shen, Y., Liang, X., 2016. Evaluation of COD effect on anammox process and microbial communities in the anaerobic baffled reactor (ABR). Bioresour. Technol. 216, 571–578.

Chen, X., Ni, B.J., Sin, G., 2019. Nitrous oxide production in autotrophic nitrogen removal granular sludge: a modeling study. Biotechnol. Bioeng. 116, 1280–1291.

Chung, Y.-C., Chung, M.-S., 2000. BNP test to evaluate the influence of C/N ratio on N_2O production in biological denitrification. Water Sci. Technol. 42, 23–27.

Conthe, M., Lycus, P., Arntzen, M.Ø., Ramos, A., Frostegård, Å., Bakken, L.R., Kleerebezem, R., van Loosdrecht, M.C.M.M., 2019. Denitrification as an N_2O sink. Water Res. 151, 381–387.

De Kreuk, M.K., van Loosdrecht, M.C.M., 2004. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci. Technol. 49, 9–17.

Domingo-Félez, C., Mutlu, A.G., Jensen, M.M., Smets, B.F., 2014. Aeration strategies to mitigate nitrous oxide emissions from single-stage nitritation/anammox reactors. Environ. Sci. Technol. 48, 8679–8687.

Gilbert, E.M., Agrawal, S., Karst, S.M., Horn, H., Nielsen, P.H., Lackner, S., 2014. Low temperature partial nitritation/anammox in a moving bed biofilm reactor treating low strength wastewater. Environ. Sci. Technol. 48, 8784–8792.

Han, M., Vlaeminck, S.E., Al-omari, A., Wett, B., Bott, C., Murthy, S., De Clippeleir, H., 2020. Uncoupling the solids retention times of flocs and granules in mainstream deammonification : a screen as effective out-selection tool for nitrite oxidizing bacteria. Bioresour. Technol. 221, 195–204.

Harris, E., Joss, A., Emmenegger, L., Kipf, M., Wolf, B., Mohn, J., Wunderlin, P., 2015. Isotopic evidence for nitrous oxide production pathways in a partial nitritation-anammox reactor. Water Res. 83, 258–270.

Hiatt, W.C., Grady Jr., C.P.L., 2008. An updated process model for carbon oxidation, nitrification, and denitrification. Water Environ. Res. 80, 2145–2156.

Hu, Z., Wessels, H.J.C.T., van Alen, T., Jetten, M.S.M., Kartal, B., 2019. Nitric oxide-dependent anaerobic ammonium oxidation. Nat. Commun. 10.

Hubaux, N., Wells, G., Morgenroth, E., 2015. Impact of coexistence of flocs and biofilm on performance of combined nitritation-anammox granular sludge reactors. Water Res. 68, 127–139.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jenni, S., Vlaeminck, S.E., Morgenroth, E., Udert, K.M., 2014. Successful application of nitritation/anammox to wastewater with elevated organic carbon to ammonia ratios. Water Res. 49, 316–326.

Jia, M., Castro-Barros, C.M., Winkler, M.K.H.H., Volcke, E.I.P., 2018. Effect of organic matter on the performance and N_2O emission of a granular sludge anammox reactor. Environ. Sci. Water Res. Technol. 4, 1035–1046.

Joss, A., Derlon, N., Cyprien, C., Burger, S., Szivak, I., Traber, J., Siegrist, H., Morgenroth, E., 2011. Combined nitritation-anammox: advances in understanding process stability. Environ. Sci. Technol. 45, 9735–9742.

Kindaichi, T., Okabe, S., Satoh, H., Watanabe, Y., 2004. Effects of hydroxylamine on microbial community structure and function of autotrophic nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 49, 61–68.

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.M., 2014. Full-scale partial nitritation/anammox experiences - an application survey. Water Res. 55, 292–303.

Lang, L., Piveteau, S., Azimi, S., Rocher, V., Spérandio, M., 2019. Modelling N_2O emission from PNA process under oxygen limitation: model calibration and prospects. 10th IWA Symposium on Modelling and Intergrated Assessment. Copenhagen, Denmark, pp. 562–565.

Laureni, M., Weissbrodt, D.G., Villez, K., Robin, O., Jonge, N.De, Rosenthal, A., Wells, G., Nielsen, J.L., Morgenroth, E., Joss, A., 2019. Biomass segregation between biofilm and flocs improves the control of nitrite-oxidizing bacteria in mainstream partial nitritation and anammox processes. Water Res. 154, 104–116.

Leal, C.D., Pereira, A.D., Nunes, F.T., Ferreira, L.O., Coelho, A.C.C., Bicalho, S.K., Mac Conell, E.F.A., Ribeiro, T.B., de Lemos Chernicharo, C.A., de Araújo, J.C., 2016. Anammox for nitrogen removal from anaerobically pre-treated municipal wastewater: effect of COD/N ratios on process performance and bacterial community structure. Bioresour. Technol. 211, 257–266.

Li, J., Zhang, L., Peng, Y., Zhang, Q., 2017a. Effect of low COD/N ratios on stability of single-stage partial nitritation/anammox (SPN/A) process in a long-term operation. Bioresour. Technol. 244, 192–197.

Li, K., Fang, F., Wang, H., Wang, C., Chen, Y., Guo, J., Wang, X., Jiang, F., 2017b. Pathways of N removal and N_2O emission from a one-stage autotrophic N removal process under anaerobic conditions. Sci. Rep. 7, 42072.

Liu, Y., Zhao, T., Su, Z., Zhu, T., Ni, B., 2020. Evaluating the roles of coexistence of sludge flocs on nitrogen removal and nitrous oxide production in a granule-based autotrophic nitrogen removal system. Sci. Total Environ. 730, 139018.

Ma, C., Jensen, M.M., Smets, B.F., Thamdrup, B., 2017. Pathways and controls of N_2O production in nitritation–anammox biomass. Environ. Sci. Technol. 51, 8981–8991.

Mozumder, M.S.I., Picioreanu, C., van Loosdrecht, M.C.M., Volcke, E.I.P., 2014. Effect of heterotrophic growth on autotrophic nitrogen removal in a granular sludge reactor. Environ. Technol. 35, 1027–1037.

Okabe, S., Oshiki, M., Takahashi, Y., Satoh, H., 2011. N_2O emission from a partial nitrification-anammox process and identification of a key biological process of N_2O emission from anammox granules. Water Res. 45, 6461–6470.

Peng, L., Ni, B., Ye, L., Yuan, Z., 2015. The combined effect of dissolved oxygen and nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. Water Res. 73, 29–36.

Picioreanu, C., Pérez, J., van Loosdrecht, M.C.M., 2016. Impact of cell cluster size on apparent half-saturation coefficients for oxygen in nitrifying sludge and biofilms. Water Res. 106, 371–382.

Pijuan, M., Ribera-guardia, A., Luís, J., Micó, M.M., de la Torre, T., 2020. Effect of COD on mainstream anammox : evaluation of process performance, granule morphology and nitrous oxide production. Sci. Total Environ. 712, 136372.

Pocquet, M., Wu, Z., Queinnec, I., Spérandio, M., 2016. A two pathway model for N_2O emissions by ammonium oxidizing bacteria supported by the NO/N_2O variation. Water Res. 88, 948–959.

Reichert, P., Ruchti, J., Simon, W., 1996. AQUASIM: Computer program for the identification and simulation of aquatic systems. Hydroinformatics '96. Vols 1 and 2. A a Balkema, Rotterdam.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.

Schulthess, Rv, Wild, D., Gujer, W., 1994. Nitric and nitrous oxides from denitrifying activated sludge at low oxygen concentration. Water Sci. Technol. 30, 123.

Su, Q., Domingo-Félez, C., Jensen, M.M., Smets, B.F., 2019. Abiotic nitrous oxide (N_2O) production is strongly pH dependent, but contributes little to overall N_2O emissions in biological nitrogen removal systems. Environ. Sci. Technol. 53, 3508–3516.

Van Dongen, U., Jetten, M.S.M., van Loosdrecht, M.C.M., 2001. The SHARON®-Anammox® process for treatment of ammonium rich wastewater. Water Sci. Technol. 44, 153–160.

Vlaeminck, Siegfried E., Terada, A., Smets, B.F., De Clippeleir, H., Schaubroeck, T., Bolea, S., Demeestere, L., Mast, J., Boon, N., Carballa, M., Verstraete, W., 2010. Aggregate size and architecture determine microbial activity balance for one-stage partial nitritation and anammox. Appl. Environ. Microbiol. 76, 900–909.

Volcke, E.I.P., Picioreanu, Cristian, De Baets, B., van Loosdrecht, M.C.M., 2010. Effect of granule size on autotrophic nitrogen removal in a granular sludge reactor. Environ. Technol. 31, 1271–1280.

Volcke, E.I.P., Picioreanu, C., De Baets, B., van Loosdrecht, M.C.M., 2012. The granule size distribution in an anammox-based granular sludge reactor affects the conversion-implications for modeling. Biotechnol. Bioeng. 109, 1629–1636.

Wan, X., Baeten, J.E., Volcke, E.I.P., 2019. Effect of operating conditions on N_2O emissions from one-stage partial nitritation-anammox reactors. Biochem. Eng. J. 143, 24–33.

Wan, X., Baeten, J.E., Laureni, M., Volcke, E.I.P.P., 2021. Ammonium-based aeration control improves nitrogen removal efficiency and reduces N_2O emissions for partial nitritation-anammox reactors. Chemosphere 274, 129720.

Wett, B., Omari, A., Podmirseg, S.M., Han, M., Akintayo, O., Gómez Brandón, M., Murthy, S., Bott, C., Hell, M., Takács, I., Nyhuis, G., O'Shaughnessy, M., 2013. Going for mainstream deammonification from bench to full scale for maximized resource efficiency. Water Sci. Technol. 68, 283–289.

Wrage, N., Velthof, G.L., Van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33, 1723–1732.

Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L., Siegrist, H., 2012. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. Water Res. 46, 1027–1037.

Wyffels, S., Van Hulle, S.W.H., Boeckx, P., Volcke, E.I.P., Van Cleemput, O., Vanrolleghem, P.A., Verstraete, W., 2004. Modeling and simulation of oxygen-limited partial nitritation in a membrane-assisted bioreactor (MBR). Biotechnol. Bioeng. 86, 531–542.

Zhou, Y., Pijuan, M., Zeng, R.J., Yuan, Z., 2008. Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. Environ. Sci. Technol. 42, 8260–8265.