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# Ammonium-based aeration control improves nitrogen removal efficiency and reduces $N_2O$ emissions for partial nitritation-anammox reactors

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## HIGHLIGHTS

- Aeration control and floc abundance impact N removal and $N_2O$ emissions.
- Constant $Q_{air}$ or effluent $NH_4^+$ control suppresses NOB at varying floc content, constant DO do not.
- Sharp drops in floc fractions cause $N_2O$ peaks at constant Qair and $NH_4^+$ control.
- Effluent $NH_4^+$ control led to the highest N removal and the lowest $N_2O$ emissions.

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## ABSTRACT

This study deals with the effect of aeration control strategies on the nitrogen removal efficiency and nitrous oxide ($N_2O$) emissions in a partial nitritation–anammox reactor with granular sludge. More specifically, dissolved oxygen (DO) control, constant airflow and effluent ammonium ($NH_4^+$) control strategies were compared through a simulation study. Particular attention was paid to the effect of flocs, which are deliberately or unavoidable present besides granules in this type of reactor. When applying DO control, DO setpoints had to be adjusted to the amount of flocs present in the reactor to maintain high nitrogen removal and reduce $N_2O$ emissions, which is difficult to realize in practice because of variable floc fractions. Constant airflow rate control could maintain a good nitrogen removal efficiency independent of the floc fraction in the reactor, but failed in $N_2O$ mitigation. Controlling aeration based on the effluent ammonium concentration results in both high nitrogen removal and relatively low $N_2O$ emissions, also in the presence of flocs. Fluctuations in floc fractions caused significant upsets in nitrogen removal and $N_2O$ emissions under DO control but had less effect at constant airflow and effluent ammonium control. Still, rapid and sharp drops in flocs led to a peak in $N_2O$ emissions at constant airflow and effluent ammonium control. Overall, effluent ammonium control reached the highest average nitrogen removal and lowest $N_2O$ emissions and consumed the lowest aeration energy under fluctuating floc concentrations.



## 1. Introduction

Partial nitritation-anammox (PNA) technologies can remove nitrogen (N) from wastewater with considerable savings in energy, external organic carbon, and sludge treatment compared to conventional nitrification-denitrification processes (van Dongen et al., 2001). During partial nitritation, nearly half of the influent ammonium ($NH_4^+$) is oxidized to nitrite ($NO_2^-$) by aerobic Ammonia Oxidizing Bacteria (AOB). The formed $NO_2^-$ and the residual ammonium are simultaneously converted to nitrogen gas ($N_2$) by Anammox bacteria (AN). Nitrite Oxidizing Bacteria (NOB) need to be suppressed, as they can oxidize $NO_2^-$ to nitrate ($NO_3^-$), which cannot be removed via the anammox reaction (van Dongen et al., 2001). A biomass retention is required because of the slow growth of anammox bacteria (Strous et al., 1998). The partial nitritation and anammox reactions are mostly realized in a single reactor, i.e. 'one-stage', often with biomass grown in the form of

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granules (Lackner et al., 2014), as it provides long sludge retention times via its excellent settling characteristics (Vlaeminck et al., 2010).

Even though most of the biomass in granular sludge partial nitritation-anammox reactors are actual granules, up to 43% of the total VSS mass can be present as flocculent sludge with a diameter below 0.1 mm (Vlaeminck et al., 2008). The microbial populations distribute differently in different aggregate size fractions. For example, anammox bacteria mainly resides in granules while AOB and NOB dominate the smaller granules and flocs (Bin et al., 2011; Corbalá-Robles et al., 2016; Hubaux et al., 2015; Vlaeminck et al., 2010; Volcke et al., 2012). Several studies, therefore, suggested selective wasting of flocs as an efficient way to suppress NOB (Han et al., 2020; Hubaux et al., 2015; Laureni et al., 2019). However, floc removal requires additional separation devices like screen (Han et al., 2020) or cyclone (Wett et al., 2013) and flocs removal may be hampered as floccular sludge in some systems have been observed attached to granules (Pijuan et al., 2020), thus potentially leading to more or less active flocs coexistence with granules in the reactor. Besides, the aggregate size distribution varies between different reactors (Shi et al., 2016). Even for the same reactor, the amount of flocs can change over time (Pijuan et al., 2020; Shi et al., 2016). Changing in operating conditions such as implement of cyclone (Wett et al., 2013) or sieve (Han et al., 2020), varying hydrodynamic conditions (Arrojo et al., 2006) and influent compositions (Han et al., 2020; Pijuan et al., 2020), can also led to fluctuations in the amount of flocs.

The aeration flow rate in a partial nitritation-anammox reactor is typically controlled with a fixed dissolved oxygen (DO) setpoint (Lackner et al., 2014). However, the optimal DO setpoint for maximum nitrogen removal depends on the aggregate size distribution (Volcke et al., 2012). For example, when more flocs are present, lower DO setpoints are needed to maximize nitrogen removal (Corbalá-Robles et al., 2016; Hubaux et al., 2015). As a result, it is difficult to maintain the DO setpoint optimal under realistic conditions, with fluctuating aggregate size distributions. If a constant DO concentration is used, a higher concentration of flocs allows the accumulation of NOB, which hampers nitrogen removal. This is explained by the less mass transfer limitation and thus higher DO concentrations inside flocs compared to granules, which favour NOB (Corbalá-Robles et al., 2016; Hubaux et al., 2015).

The emissions of $N_2O$, a potent greenhouse gas, are also affected by the aeration control and DO. $N_2O$ can be produced biologically during the ammonium oxidation in two pathways: 1) nitrifier nitrification pathway, where $NH_2OH$, an intermediate during ammonium oxidation, is oxidized to NO with $N_2O$ as a by-product; 2) nitrifier denitrification, which involves the reduction of nitrite to $N_2O$ (Wunderlin et al., 2012). Besides, $N_2O$ is an obligatory intermediate during heterotrophic denitrification and can be produced or consumed in this process (Knowles, 1982). The contribution of the $N_2O$ production pathways varies under different DO (Ma et al., 2017). The DO setpoints that lead to high nitrous oxide ($N_2O$) emissions also depend on the aggregate size distribution. For example, high $N_2O$ emissions are obtained at higher DO values when the granule size increases (Wan et al., 2019, simulation study). Moreover, a more heterogeneous granule size distribution (Chen et al., 2019b, simulation study) or a higher floc fractions (Liu et al., 2020, simulation study) increase $N_2O$ emissions when a fixed DO setpoint is applied. The question arises whether a different aeration control strategy, with dynamic DO setpoint, could improve nitrogen removal and reduce $N_2O$ emissions under realistic conditions with dynamic, heterogeneous aggregation.

Apart from DO control, many alternative aeration control strategies have been applied in the one-stage partial nitritation anammox reactors (Lackner et al., 2014). The aeration is controlled via a patented method using the ratio of influent and effluent ammonium concentration in ANITAMox™ MBBRs. Constant aeration airflow is also applied in some lab-scale reactors (Sliekers et al., 2002). There are also many studies evaluating different aeration control strategies for partial nitritation anammox reactors. Joss et al. (2011) suggested using volumetric air flow rates instead of DO as a proxy for $O_2$ supply in combination with AOB activity to reduce the nitrite accumulation in the reactor. Schraa et al. (2020) compared controlling aeration of a mainstream PNA system with a fixed DO setpoint, intermittent aeration and effluent ammonium control, in which DO setpoint was regulated by effluent ammonium. The simulation indicated that effluent ammonium control provides the best compromise between removing nitrogen and minimizing energy consumption. Klaus et al. (2017) applied pH-based aeration control in a one-stage partial nitritation anammox reactor and obtained a stable nitrogen removal. Al-Omari et al. (2015) reported that ammonium/$NO_x$ control, maintaining a certain ratio between ammonium and $NO_x$ by controlling aerobic duration, was more efficient in suppressing NOB than control based on ammonium alone. Aeration control strategies also affect $N_2O$ emissions (Castro-Barros et al., 2015). For example, an experimental study (Domingo-Félez et al., 2014) reported that frequently switching aeration on and off to shorten the aeration time reduces $N_2O$ emissions. All the above mentioned studies use either only nitrogen removal or only $N_2O$ emissions as an evaluating indicator and the effects of flocs and its fluctuations on both nitrogen removal and $N_2O$ emissions were never investigated when evaluating the aeration control strategies.

This study evaluated the effect of the aeration control strategy on the nitrogen removal and $N_2O$ emissions in partial nitration-anammox systems with different and fluctuating concentrations of flocs coexisting with the granular biomass. Three aeration control strategies were simulated, namely DO control, constant airflow and effluent ammonium control, to improve the nitrogen removal efficiency while minimizing $N_2O$ emissions over a wide range of floc/granule ratios.

## 2. Materials and methods

An existing 1D biofilm model set-up in the software Aquasim (Reichert, 1994) was applied to simulate a one-stage granular sludge PNA reactor (supplementary information S1). Bioconversions including $N_2O$ formation were described through well-established models from literature, including the associated parameter values. The metabolism of AOB including $N_2O$ production pathways was based on Pocquet et al. (2016). Heterotrophic denitrification with $N_2O$ as an intermediate was described as in Hiatt and Grady (2008). The metabolisms of NOB and anammox bacteria were taken from Mozumder et al. (2014). The stoichiometric matrix, process rates, and applied parameters are summarized in Table S2, Table S3 and Table S4, respectively.

The model was simulated for an influent flow rate of 2500 $m^3$ $d^{-1}$, containing 300 g N. $m^{-3}$ ammonium and 150 g COD.$m^{-3}$. A total reactor volume of 400 $m^3$ was assumed, including 300 $m^3$ of perfectly mixed bulk liquid and 100 $m^3$ of granular sludge. A characteristic granule diameter of 1.5 mm was used (Kampschreur et al., 2009). Details on the reactor configuration and granule characteristics were included in Table S5.

The fraction of flocs (mass % of total biomass present as flocs) that coexists with the granular biomass was determined (Table S7) by varying the floc retention factor, i.e. the fraction of flocs detached from the granules that were retained in the reactor. The retention of flocs was modelled by implementing a bifurcation from the effluent into the inlet, as described by Hubaux et al. (2015).

The reactor performance, in terms of nitrogen removal and $N_2O$

emissions, was evaluated under different floc retention factors (from 0 to 0.99, corresponding to different floc fractions shown in Table S7), through steady-state simulations, considering three types of aeration control strategies. In the first strategy, the aeration flow rate was manipulated with a PI feedback controller to keep the DO constant (control strategy explained in the supplementary information, section S1.3.1). Besides, a series of simulations at different DO setpoints between 0.3 and 1.8 g $O_2$.m$^{-3}$ was performed (with a resolution of 0.1 g $O_2$. m$^{-3}$) for each floc retention factor to find the optimal DO for nitrogen removal at different floc fractions. In the second aeration strategy, the airflow rate was kept at a constant level. The third control strategy consisted of a cascade controller in which the DO setpoint was determined by a master controller maintaining a constant effluent ammonium concentration. In order to meet this DO setpoint, the aeration flowrate was adjusted by a slave controller. Both master and slave controllers were of the PI type. The maximum nitrogen removal efficiency without floc retention was obtained at a DO of 0.9 g $O_2$. m$^{-3}$, corresponding with an airflow rate $Q_{air}$ = 25600 m$^{-3}$. d$^{-1}$ and an effluent ammonium concentration of 14 g N. m$^{-3}$ (Figure S1). Therefore, these values were taken as setpoints for the different aeration control strategies, also in the presence of flocs.

Besides steady-state simulations, two series of dynamic simulations were conducted to evaluate the above-mentioned aeration control strategies under fluctuating floc retention factors (Table 1). First, sudden drops of floc retention factor (from 0.99 to 0 and from 0.92 to 0) were simulated under different aeration control strategies to understand the effect of fluctuations on the nitrogen removal and $N_2O$ emissions. In case of DO control, the DO setpoints were set to 0.2 and 0.6 g $O_2$. m$^{-3}$, corresponding to the optimal N nitrogen removal at the different initial floc retention factor, namely 0.99 and 0.92, respectively. The corresponding airflow rates ($Q_{air}$ 23300 and 24600 m$^{-3}$. d$^{-1}$, respectively), and effluent ammonium concentrates (12.5 and 14 g N. m$^{-3}$, respectively) were chosen as setpoints for constant airflow and effluent ammonium control strategies. The three aeration control strategies were evaluated under randomly fluctuating floc retention factors (Table S9), varying floc fractions between 0.3% and 42% of the total biomass (Vlaeminck et al., 2008), with a maximum changing rate of around 8% per day (Han et al., 2020). In this way, the cumulative nitrogen removal and $N_2O$ emissions could be compared for the three control strategies during reasonably expected fluctuations of flocs. The setpoints were chosen the same as that in steady-state simulations. For all dynamic simulations, the model was first simulated for 5000 days without floc retention (floc retention factor: 0) to ensure a steady-state and followed by simulations with dynamic floc retention factors.

## 3. Results and discussion

### *3.1. Constant DO failed to suppress NOB and mitigate $N_2O$ emissions at high floc concentrations*

When controlling the DO at a fixed concentration of 0.9 g $O_2$.m$^{-3}$, an increasing floc fraction led to a decreasing nitrogen removal efficiency (Fig. 1 A). This decrease became steeper beyond a floc fraction of 4%, eventually leading to a nitrogen removal of 27% for a floc fraction of 19%. The initial slow decrease of the nitrogen removal can be explained by the increasing AOB concentration, especially in the flocs (Fig. 2A–B). The higher presence of AOB in flocs, where mass transfer resistance is limited, increased the aerobic oxidation of ammonium. This caused less anammox activity and thus a drop of the nitrogen removal. Once the floc fraction increased beyond 4%, NOB started to proliferate as well, both in flocs and granules, as found in earlier studies (Corbalá-Robles et al., 2016; Hubaux et al., 2015). Also, this is explained by the low mass transfer limitation and thus higher DO concentration in the flocculent biomass compared to granular biomass. The competition of NOB for nitrite with anammox led to a faster drop in nitrogen removal and in the anammox population in the granules, leading to less nitrogen removal. Interestingly, the amount of AOB in the granules increased after NOB started to grow, as the lower anammox rate left more ammonium available for AOB. An increased floc fraction also made heterotrophic populations shifting from granules to flocs, due to the limited mass transfer resistance for both COD and oxygen in flocs.

The oxygen transfer rate (OTR), increased with increasing floc fractions in the reactor (Fig. 3). A higher floc fraction both decreases mass transfer limitations and leads to a higher concentration of AOB, NOB and − to a lesser extent − heterotrophs. For a fixed DO, these higher biomass concentrations and low mass transfer limitations increase the oxygen consumption rate. The oxygen consumption rate significantly increased when NOB started to accumulate, as NOB compete for nitrite with anammox and more ammonium and nitrite are oxidized by oxygen rather than converted to $N_2$ anaerobically. To maintain the DO constant with a higher consumption rate, the controller increases the aeration rate and therefore the oxygen transfer rate also increases. As such, this control strategy leads to over aeration at high floc fractions. Consequently, the optimal DO setpoint for nitrogen removal decreased from 0.9 g $O_2$.m$^{-3}$ to 0.2 g $O_2$.m$^{-3}$ when the floc fraction increased from 0.3% to 19% (Fig. 4). This is in line with the lower optimal DO for a smaller average granule size found in other studies (Mozumder et al., 2014; Volcke et al., 2010). As flocs grow unavoidably in a reactor (Vlaeminck et al., 2008), and the floc fraction can vary over time (Arrojo et al., 2006; Shi et al., 2016),

**Table 1**
Scenarios for dynamic simulations.

| Control strategies | Initial floc retention factor at steady-state | Initial flocculent sludge fraction | Dynamics of floc retention factors in the studied periods |
|---|---|---|---|
| DO control (0.2 g $O_2$.m$^{-3}$) | 0.99 | 19% | (Sudden drop from initial values to 0) |
| DO control (0.6 g $O_2$.m$^{-3}$) | 0.92 | 3.5% | |
| Constant Qair (23300 m$^3$ d$^{-1}$) | 0.99 | 19% | |
| Constant Qair (24600 m$^3$ d$^{-1}$) | 0.92 | 3.7% | |
| Effluent $NH_4^+$ control (12.5 g N.m$^{-3}$) | 0.99 | 19% | |
| Effluent $NH_4^+$ control (14 g N.m$^{-3}$) | 0.92 | 3.7% | |
| DO control (0.9 g $O_2$.m$^{-3}$) | 0 | 0.3% | (fluctuating between 0 and 0.999) |
| Constant Qair (25600 m$^3$ d$^{-1}$) | 0 | 0.3% | |
| Effluent $NH_4^+$ control (14 g N.m$^{-3}$) | 0 | 0.3% | |

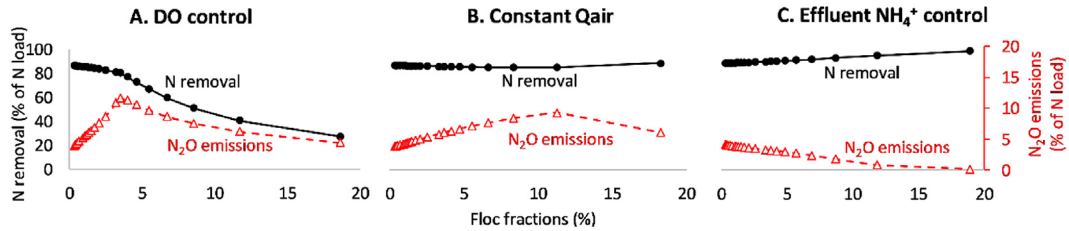


**Fig. 1.** N removal and $N_2O$ emissions as a function of floc fractions under different aeration control strategies. Results were obtained with setpoints optimized for the case without floc retention, namely a DO setpoint of 0.9 $gO_2.m^{-3}$ (A), airflow rate $Q_{air}$ = 25 600 $m^3$ $d^{-1}$ (B) and an effluent ammonium concentration of 14 g $NH_4^+$-N. $m^{-3}$ (C).

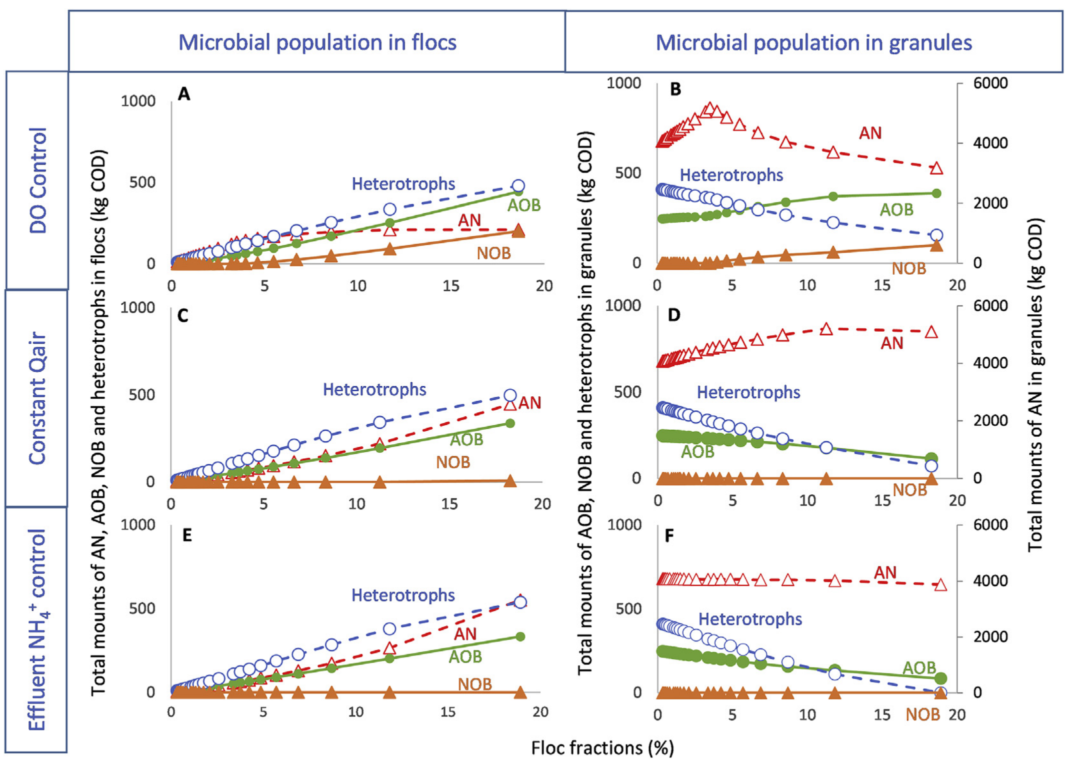


**Fig. 2.** Total amount of microbial populations located in flocs (left: A, C, E) and granules (right: B, D, F) as a function of floc fractions. Results for different aeration control strategies: (A, B) DO control; (C, D) constant airflow; (E, F) effluent $NH_4^+$ control.

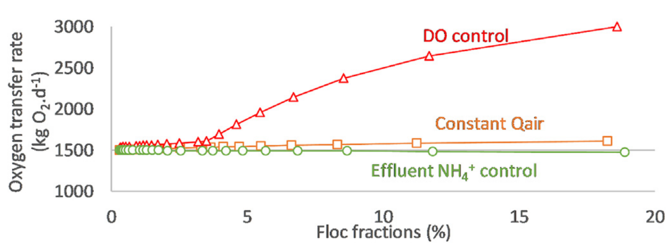


**Fig. 3.** Oxygen transfer rate (OTR) as a function of floc fractions at different aeration control strategies.

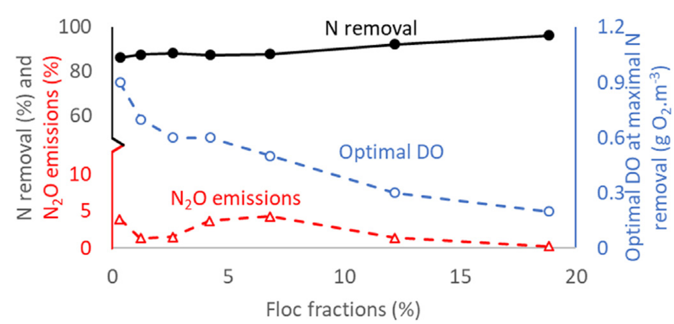


**Fig. 4.** Optimal DO setpoints and the corresponding nitrogen removal efficiency and $N_2O$ emissions for maximal nitrogen removal as a function of floc fractions.

aeration control with a fixed DO setpoint requires regular manual adjustment of the setpoint. Selectively biomass retention with a hydro-cyclone enables wash-out of NOB and improves nitrogen removal (Wett et al., 2013), but requires additional operating costs.

The floc fraction in a partial nitritation-anammox reactor also affects $N_2O$ emissions (Fig. 1): with a fixed DO setpoint of 0.9 g $O_2$. $m^{-3}$, $N_2O$ emissions first increased with higher floc fractions (0.3–4%) and then declined after a peak (Fig. 1A). The nitrifier denitrification pathway was the dominating $N_2O$ production pathway in all cases (Fig. 5A and B). In the model for this pathway, $N_2O$ was formed from the combination of $NH_2OH$ oxidation and nitrite in equimolar amounts (process 4 in Table S2). More specifically, 50–52% of total $N_2O$ production was derived from

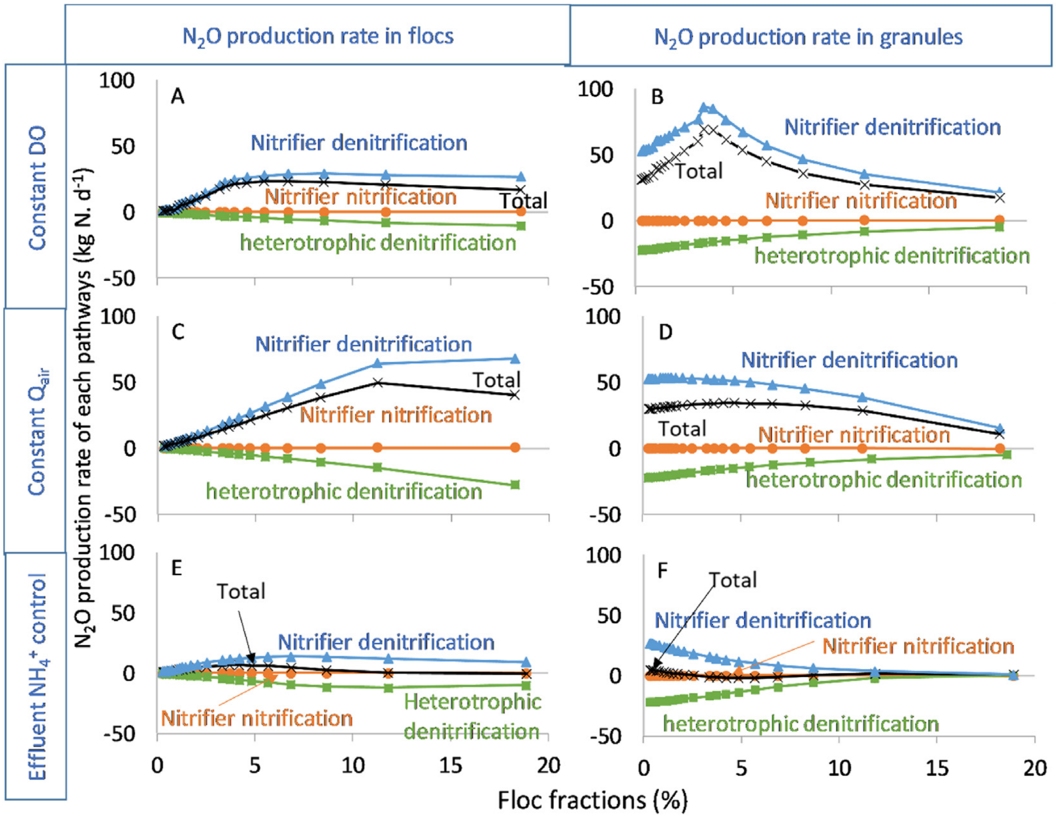


**Fig. 5.** Rate of $N_2O$ production pathways in flocs (left: A, C, E) and granules (right: B, D, F) as a function of floc fractions. Results for different aeration control strategies: DO control (A, B); constant airflow (C, D); effluent $NH_4^+$ control (E, F).

hydroxylamine oxidation while nitrite reduction contributed 48%—50% to the total $N_2O$ production. This is in line with experimental reports that $NH_2OH$ oxidation (50%—55%) and nitrite reduction (45% —55%) contributed roughly equal to $N_2O$ production in a similar reactor (Ali et al., 2016).

The increasing $N_2O$ emissions with increasing floc fractions up to 4% were explained by the increased nitrite production by AOB (nitrite concentration increased from 1.2 to 1.8 g N.$m^{-3}$, Figure S2), leading to an increased $N_2O$ production by nitrifier denitrification pathway in both flocs and granules (Fig. 5A and B). Indeed, nitrite was the limiting substrate for nitrifier denitrification pathway ($K_{AOB,NO2}$: 20.2 g N.$m^{-3}$, Table S4). The decreasing concentration of $NH_2OH$ as an additional substrate (from 1.8 to 1.6 g N.$m^{-3}$) was not significant as it was not limiting ($K_{AOB,NH2OH}$: 0.9 g N.$m^{-3}$). For floc fractions higher than 4%, NOB started to grow in both flocs and granules (Fig. 2 A and B), consuming nitrite (nitrite concentration decreased from 1.8 to 0.2 g N.$m^{-3}$, Figure S2) and thus leading to decreased $N_2O$ production by nitrifier denitrification.

Heterotrophic denitrification was a sink of $N_2O$ in this system, indicating that the heterotrophic $N_2O$ production rate ($NO_2^-$ to $N_2O$, process 13 and 14, Table S2) was lower than the heterotrophic $N_2O$ consumption rate ($N_2O$ to $N_2$, process 15, Table S2). $N_2O$ formation by heterotrophic denitrification was limited by the low NO concentration (around $9.8 \times 10^{-4}$ g N. $m^{-3}$, about 50 times lower than the affinity constant of heterotrophs: $K_{Het,NO} = 0.05$ g N. $m^{-3}$). This low NO concentration could have been caused either by its consumption by AOB (in processes 3 and 5, Table S2; $K_{AOB,HAO,NO} = 0.0003$ g N. $m^{-3}$ and $K_{AOB,NN,NO} = 0.008$ g N. $m^{-3}$) or by limited NO production from nitrite by heterotrophs due to the competition for nitrite with anammox ($K_{AN,NO2} = 0.005$ g N. $m^{-3}$ compared to $K_{Het,NO2} = 0.2$ g N. $m^{-3}$). Likely the latter was the main reason for the low $N_2O$ formation by heterotrophic denitrification in the one-stage partial nitritation anammox reactors. In nitrifying-denitrification systems where anammox is not present, NO production from heterotrophs by nitrite is not limited by the competition for nitrite and heterotrophic denitrification has been reported as a strong $N_2O$ source, under COD limiting conditions preventing further $N_2$ production (Itokawa et al., 2001; Kester et al., 1997). This was also confirmed by simulation studies for nitrifying-denitrification systems where the competition for NO by AOB did not make heterotrophs a $N_2O$ sink (Domingo-Félez et al., 2017; Ni et al., 2015). Besides, the high nitrogen load in this reactor can lead to high $N_2O$ concentrations in the liquid phase (up to 2.1 g N.$m^{-3}$), which stimulated the $N_2O$ consumption by heterotrophic denitrification.

Heterotrophic denitrification was also reported as an $N_2O$ sink in other simulation studies for one-stage partial nitritation-anammox reactors (Chen et al., 2019a; Lang et al., 2019; Wan et al., 2019). Experimental evidence of heterotrophic denitrification being a net sink in this system has not been provided so up till now. Still, the net consumption of $N_2O$ was observed in the anoxic period of both full-scale (Castro-Barros et al., 2015) and lab-scale (Domingo-Félez et al., 2014; Yang et al., 2016) reactors with intermittent aeration, which were postulated to be caused by heterotrophic denitrification. A lab-scale experiment (Li et al., 2017) reported that the heterotrophic denitrification contributed 64% of $N_2O$ emissions, but this value was obtained from a batch test with high nitrite concentration (100 g N.$m^{-3}$), which is not a representative situation for full-scale systems. Still, further experimental studies are required to confirm that heterotrophic denitrification indeed served as a sink of $N_2O$ in one-stage partial nitritation anammox systems.

Overall, the $N_2O$ consumption by heterotrophs did not outweigh the $N_2O$ production by nitrifier denitrification, which dominated the $N_2O$ emission pattern. The $N_2O$ emission varied between 4.0% and 12% of the influent nitrogen load over the studied range of floc fractions, when keeping the DO constant at 0.9 g $O_2$. $m^{-3}$ — which is the optimal DO setpoint in case there is hardly any floc present (Figure S1). $N_2O$ emissions could be kept lower without sacrificing nitrogen removal at floc fractions below 4.3% by lowering the DO setpoint manually (Fig. 4), but this is difficult in practice with fluctuating floc fractions.

### 3.2. Constant airflow rate allows high nitrogen removal even at high flocs but failed in $N_2O$ mitigation

Keeping the airflow rate fixed at its optimal value in case of no floc retention ($Q_{air}$ = 25600 $m^{-3}$. $d^{-1}$), the nitrogen removal efficiency remained high at all times. The nitrogen removal efficiency dropped only very slightly, from 86% to 85% for a floc fraction increasing from 0.3% up to 11% and then increased, up to 88% for a floc fraction of 19% (Fig. 1 B). The slight drop in nitrogen removal can be explained by the competition for ammonium between AOB and anammox bacteria. A higher amount of flocs amount increased the population of AOB, anammox bacteria and heterotrophs, especially in flocs (Fig. 2C and D). However, NOB did not accumulate at high floc fractions at constant airflow rate, as the increased AOB and heterotrophs concentration led to more oxygen consumption and thus a lower DO (Figure S2 B), which suppressed NOB growth. Even though the anammox bacteria concentration in flocs also increased, their activity was inhibited by the higher oxygen concentration in flocs and suppressed by the higher amounts of AOB, which compete for ammonium. Therefore, more flocs led to less anammox activity and less nitrogen removal. Once the floc fraction increased beyond 11%, the DO became so low (0.49 g $O_2$.$m^{-3}$) that anammox bacteria in the flocs were inhibited less (inhibition factor $K_{AN,I,O}$: 0.05 g $O_2$.$m^{-3}$), which led to an increasing nitrogen removal efficiency.

Although a constant airflow successfully suppressed NOB and maintained high nitrogen removal efficiencies at all studied floc fractions, $N_2O$ emissions were still high at higher floc fractions. Keeping the airflow rate constant ($Q_{air}$ = 25600 $m^{-3}$. $d^{-1}$), $N_2O$ emissions first increased with higher floc fractions (0.3–11%) and then started to decline after a peak (Fig. 1B). The increasing $N_2O$ emissions up to 11% can be explained by the increased ammonium oxidation by AOB, leading to the nitrite accumulation (from 1.27 to 1.38 g N.$m^{-3}$, Figure S2 B) and stimulating $N_2O$ production by nitrifier denitrification pathways in both flocs and granules (Fig. 5C and D). The increase of $N_2O$ production in flocs is more pronounced as fewer anammox bacteria were presented in flocs (Fig. 2), allowing more nitrite available for AOB. An increasing floc fraction also led to the gradual decrease of the bulk DO concentration (from 0.9 to 0.3 g $O_2$. $m^{-3}$). The lower DO at high floc fractions alleviated the DO inhibition on anammox bacteria, allowing them to convert more nitrite (nitrite concentration decreased from 1.38 to 1 g N. $m^{-3}$), thus reducing the $N_2O$ production by nitrifier denitrification. Similar to the DO control, nitrifier denitrification was the dominating pathway and heterotrophs served as a sink (Fig. 6 C and D).

Even though the airflow rate was constant, the oxygen transfer rate slightly increased with increasing floc fractions (Fig. 3) as lower bulk DO concentration (Figure S2 B) increased the oxygen transfer from gas phase to liquid phase (Eq. S5). Yet the oxygen transfer rate at a constant airflow rate was much lower than that in DO control, as the airflow rate was kept constant independent to the increase of oxygen consumption caused by more flocs.

### 3.3. Effluent ammonium control increases nitrogen removal efficiency and reduce $N_2O$ emissions even at high floc fractions

When aeration was controlled to maintain a constant effluent ammonium concentration (14 g N.$m^{-3}$), the nitrogen removal efficiency increased continuously from 86% to 96% with floc fractions increasing from 0.3 to 19% (Fig. 1C). The increasing nitrogen removal efficiency could be explained by the higher $N_2$ production by anammox with more flocs. Similar to the constant airflow rate control, more flocs led to more AOB, anammox bacteria and heterotrophs in flocs while NOB was successfully suppressed (Fig. 2 E and F). More flocs reduced the DO concentration at this control strategies, as flocculent AOB have higher apparent oxygen affinity and required lower bulk DO (DO reduced from 0.9 to 0.2 g $O_2$. $m^{-3}$, Figure S2 C) to reach the effluent ammonium setpoint. The lower bulk DO stimulated the competition for ammonium by anammox and further reduced the total oxygen consumption by AOB, leading to decreasing OTR (from 1494 to 1471 kg $O_2$. $d^{-1}$, Fig. 3). In addition, the DO decreased significantly at higher flocs (Figure S2 C) and thus alleviated the oxygen inhibition of anammox bacteria in the flocs even more as with constant airflow. As a result, nitrogen removal efficiency increased with higher floc concentrations this time. Another study, where DO control, intermittent aeration, and effluent ammonium control were compared, also reported that effluent ammonium control was more efficient in suppressing NOB and improving nitrogen removal (Schraa et al., 2020).

For this control strategy, the $N_2O$ emissions decreased continuously from 4% to 0.17% of the nitrogen load when the floc fraction increased from 0.3 to 19%. This can be explained by the restricted aerobic ammonium oxidation and elevated anammox activity at higher flocs, resulting in lower nitrite concentrations (from 1.3 to 0.6 g $NO_2^-$ N. $m^{-3}$, Figure S2 C) and thus decreased the $N_2O$ production by nitrifier denitrification (Fig. 5 E and F), the dominating $N_2O$ production pathway. Another study with a lab-scale experiment also suggested that more anaerobic compared to aerobic ammonium oxidation can reduce the $N_2O$ production in one-stage partial nitritation anammox reactors (Domingo-Félez et al., 2014).

Compared to the other two aeration control strategies, effluent ammonium control could not only suppress NOB and reach higher nitrogen removal efficiency but also reduced $N_2O$ emissions, independent to the amount of flocs in the reactor. Besides, effluent ammonium control also reduced energy consumption, as it resulted in lower aeration airflow rates (Figure S3).

### 3.4. Aeration control strategies affect both nitrogen removal and $N_2O$ emissions dynamics under fluctuating floc fractions

The performance of the different aeration strategies under dynamic conditions was assessed by simulating the impact of fluctuations in the floc concentration in the reactor under study. The nitrogen removal and $N_2O$ emissions showed different dynamics with fluctuating floc fractions in the reactor (Fig. 6).

Rapid floc wasting from a reactor with a high initial floc concentration (from 19% to 0.3% of total biomass) significantly reduced nitrogen removal efficiency (from more than 90% to around 10%, 45%, and 42% for DO control, constant airflow rate and effluent ammonium control, respectively) with all aeration control strategies (Fig. 6 A, B and C). This is mainly explained by the loss of the large AOB population that resided in flocs, causing a drop in the ammonium oxidation rate and nitrogen removal efficiency. At DO control, the low nitrogen removal at the sharp drop in floc content cannot be recovered until the floc content gradually increased again (Fig. 6 A, after day 100). However, at constant airflow rate and effluent ammonium control, the nitrogen removal was recovered in a few days.

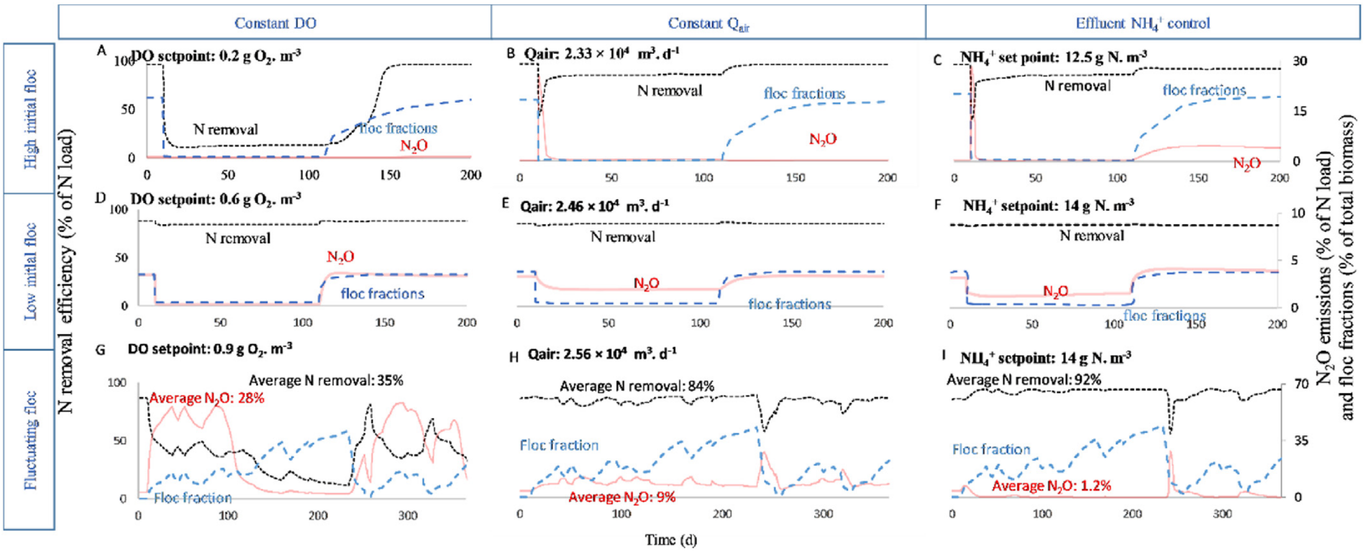


**Fig. 6.** $N_2O$ emissions and N removal dynamics upon a sudden drop in floc fraction and gradually increasing floc fraction (from reactors with high initial floc fractions: A, B and C; from reactors with low initial floc fractions: D, E, and F) and for fluctuating floc fractions (G, H and I). Results for different aeration control strategies: DO control (A, D, G); constant airflow (B, E, H); effluent $NH_4^+$ control (C, F, I).

At DO control, floc removal made more COD available for granules and stimulated the growth of heterotrophs in granules (Figure S4 A and Figure S6 B1). AOB in granules were outcompeted by the fast-growing heterotrophs, which also have a higher oxygen affinity (i.e., a lower half-saturation constant $K_{H,O} = 0.1$ g $O_2$.m$^{-3}$ versus $K_{AOB,O,1} = 1$ g $O_2$.m$^{-3}$). The suppression of AOB (Figure S4 A and Figure S6 B1) led to low nitrogen removal after floc removal (Fig. 6 A). When the floc fraction increased again (from day 100), heterotrophs decreased quickly in granules as more COD was consumed in flocs, which allowed more growth of AOB and anammox bacteria in granules (Figure S4 A). As a result, the nitrogen removal increased again with elevated AOB concentration in both granules and flocs.

When applying a constant airflow rate or effluent ammonium control, floc removal significantly increased the bulk DO concentration as most oxygen consumers (heterotrophs and AOB) were removed with flocs. The high DO concentration inhibited the anammox in granules, as the initial AOB and heterotrophs concentrations in the granules were too low (Fig. 2 D and F) to prevent oxygen from penetrating into the deep layer (Figure S5 C3 and D3) for these control strategies. Therefore, the inhibited anammox and lower ammonium oxidation (due to less AOB) together caused a drop in nitrogen removal after floc removal. The nitrogen removal recovered in a few days already (Fig. 6 B and C) as AOB and heterotrophs grew quickly in granules (Figure S4 B and C) with elevated DO and substrates concentrations. The regrowth of AOB and heterotrophs in the granules (Figure S5 C1 and D1) also decreased the oxygen penetration inside granules (Figure S6 C3 and D3) and alleviated the inhibition of oxygen on anammox.

As for $N_2O$ emissions, wasting flocs from the reactor with high initial floc fractions (floc fraction around 19%) led to nearly zero $N_2O$ emissions at DO control. As mentioned above, the growth of heterotrophs in granules outcompeted AOB (Figure S6 B1), decreasing bulk nitrite concentrations (Figure S6 B1) and the associated $N_2O$ production by AOB (Figure S6 B2). The sharp drop in floc fractions caused an $N_2O$ peak for around one week with constant airflow (Fig. 6 B) and effluent ammonium control (Fig. 6C). This is caused by the accumulation of nitrite after floc removal. As mentioned above, a decreasing floc concentration increased the bulk DO and oxygen penetration into the granules (Figure S5 C3 and D3), leading to oxygen inhibition on anammox bacteria. Due to the decreased anammox rate, nitrite accumulated in the reactor (up to 17 g N. m$^{-3}$ for constant $Q_{air}$ and 22 g N. m$^{-3}$ for effluent ammonium control) and stimulated $N_2O$ production (Figure S5 C2 and D2). After several days, AOB and heterotrophs grew in the granules (Figure S6 C1 and D1), reducing the oxygen penetration into the granules and relieving the inhibition on anammox bacteria. The regained nitrite consumption rate decreased nitrite concentration (below 1 g N. m$^{-3}$) and reduced the $N_2O$ emissions (Figure S6 C2 and D2).

In case of low initial floc fractions, floc wasting (from 3.5% to 0.3%) caused a slightly lower nitrogen removal with DO control (Fig. 6 D), as the floc removal made more heterotrophs growing in granules (Figure S4 D), competing for oxygen with AOB. As a result, the amount of AOB inside the granules slightly decreased after floc removal (Figure S4. D). However, fewer flocs had no significant effect on nitrogen removal with constant airflow and effluent ammonium control (Fig. 6 E and F) as less ammonium oxidation in flocs was compensated by higher ammonium oxidation in granules at higher DO and slightly increased AOB in granules. $N_2O$ emissions dropped at lower flocs without any $N_2O$ peak for all aeration control strategies. This was explained by the higher AOB and heterotrophs populations at granules at low initial floc fractions (Fig. 2 B, D and F), which prevented the inhibition of anammox by oxygen. Therefore, the nitrite accumulation and the consequently high $N_2O$ production were avoided.

With fluctuating floc fractions, the nitrogen removal and $N_2O$ emissions showed different dynamics at different aeration control strategies (Fig. 6 G, H and I). In case of DO control, variations in floc fractions caused drastic oscillations in nitrogen removal (standard deviation: 16%) and $N_2O$ emissions (standard deviation: 21%) under DO control. However, under constant airflow or effluent ammonium control, the nitrogen removal efficiency was relatively stable (standard deviation 6% and 5%, respectively) and so were $N_2O$ emissions (standard deviation 5% and 3%, respectively). This is because the constant $Q_{air}$ and effluent ammonium control strategies can adapt to the new floc content by automatically adjusting DO and allowing the adaption of the microbial population inside the granules to compensate for the changes in flocs. Additional simulations with high initial floc fractions (around 10%) also showed that effluent ammonium control led to better nitrogen removal and less $N_2O$ emissions (Figure S9). The sharp drop in flocs (from 42% to 4% between day 231 and day 241, Fig. 6H and I) caused a high peak in $N_2O$ emissions for constant $Q_{air}$ and effluent ammonium control, as expected from the simulations with a sudden drop from a high initial floc fraction. Therefore, the sharp drop of floc fractions could still cause $N_2O$ peaks at constant $Q_{air}$ and effluent ammonium control. This sharp drop in floc contents could happen when solid retention time decreased sharply or floc removal devices (e.g., cyclone or sieve) were applied (Han et al., 2020), which is not often in standard operation. Overall, the effluent ammonium control reached the highest (time-weighted) average nitrogen removal (92%) and the lowest average $N_2O$ emissions (1.2%). Besides, effluent ammonium control reduced the energy costs by aeration, as it required the lowest average aeration air flow rate (Table S9). Schraa et al. (2020) also reported that effluent ammonium control led to a higher nitrogen removal. However, the aeration energy consumptions between DO control and effluent ammonium control were similar in their study. One possible reason could be that the variation in flocs was not considered in their evaluation. Therefore, the increase of oxygen consumption with more flocs at constant DO was overlooked in their study.

In this study, the $N_2O$ emissions ranged from 0.2 to 12% in steady-state simulations, comparable to the reported values in literature, which varied from 0.4 to 19% of the nitrogen load depending on operating conditions and reactor configuration (Wan et al., 2019). High $N_2O$ emissions (around 35% of nitrogen load) occurred when floc content fluctuates during dynamic simulations.

It should be noted that the model in this study was not calibrated but made use of widely applied literature values. The results should therefore not be interpreted in a fully quantitative way. Even though it is clear that precise parameter values (e.g. NOB kinetics) will affect the numerical simulation outcomes (e.g. optimal DO setpoint), it can reasonably be expected that the trends and conclusions will remain the same. Nevertheless, the model remains most useful to qualitatively evaluate the effect of aeration control strategies in the presence of and under fluctuating flocs on nitrogen removal efficiency and $N_2O$ emissions, based on the current understanding of the $N_2O$ formation mechanisms, which was the aim of this study.

## 4. Conclusions

The effect of aeration control strategies on the nitrogen removal efficiency and $N_2O$ emissions was studied in a granular sludge partial nitritation−anammox reactor through simulation. Particular attention was paid to the effect of flocs. The commonly applied DO control strategy failed to suppress NOB when flocs accumulated in the reactor, resulting in lower nitrogen removal efficiencies. Furthermore, it could not keep $N_2O$ emissions low due to suboptimal DO setpoints for given floc fractions. In order to keep the nitrogen removal efficiency high and $N_2O$ emissions low, the DO setpoint would need to be lowered manually with increasing floc fractions, which is impractical.

In contrast, applying a constant airflow rate could successfully suppress NOB and maintain good nitrogen removal independently to the number of flocs. Still, it could not limit $N_2O$ emissions when flocs accumulated. Effluent ammonium control, which adjusts the DO setpoint automatically based on the effluent ammonium, led to improved nitrogen removal and reduced $N_2O$ emissions also when flocs accumulated. Besides, it could successfully suppress NOB, and reduce the operating costs due to lower aeration requirements.

Under fluctuating flocs, DO control caused significant upsets in nitrogen removal efficiency and $N_2O$ emissions. Constant airflow rate and effluent ammonium reached more stabilized nitrogen removal and $N_2O$ emissions. Still, the rapid and sharp drop in floc fractions temporarily decreased nitrogen removal efficiency and stimulated $N_2O$ emissions for several days at constant airflow and effluent ammonium control, as the distribution of microbial populations in granules needed time to adapt to the higher DO at lower floc concentrations. Under fluctuating flocs fractions, effluent ammonium control reached the highest average nitrogen removal as well as the lowest average $N_2O$ emissions and aeration requirements.

## Author contribution

Xinyu Wan: Conceptualization, Writing − original draft, Formal analysis, Methodology, Software, Review & Editing, Janis E. Baeten: Conceptualization, Software, Review & Editing, Michele Laureni: Conceptualization, 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.129720.

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