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Comparison of N₂O mitigation strategies

**Smart operation of nitrification/denitrification virtually abolishes nitrous oxide
emission during treatment of co-digested pig slurry centrate**

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ABSTRACT:

The implementation of nitrification/denitrification (Nit/DNit) as alternative to nitrification/denitrification (N/DN) is driven by operational cost savings, e.g. 1.0-1.8 EUR/ton slurry treated. However, as for any biological nitrogen removal process, Nit/DNit can emit the potent greenhouse gas nitrous oxide (N₂O). Challenges remain in understanding formation mechanisms and in mitigating the emissions, particularly at a low ratio of organic carbon consumption to nitrogen removal (COD_{rem}/N_{rem}). In this study, the centrate (centrifuge supernatant) from anaerobic co-digestion of pig slurry was treated in a sequencing batch reactor. The process removed approximately 100% of ammonium at a satisfactory nitrogen loading rate (0.4 g N/L/d), with minimum nitrite and nitrate in the

effluent. Substantial N_2O emission (around 17% of the ammonium nitrogen loading) was observed at the baseline operational condition (dissolved oxygen, DO, levels averaged at 0.85 mg O_2/L ; $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ of 2.8) with ~68% of the total emission contributed by nitrification. Emissions increased with higher nitrite accumulation and lower organic carbon to nitrogen ratio. Yet, higher DO levels (~2.2 mg O_2/L) lowered the aerobic N_2O emission and weakened the dependency on nitrite concentration, suggesting a shift in N_2O production pathway. The most effective N_2O mitigation strategy combined intermittent patterns of aeration, anoxic feeding and anoxic carbon dosage, decreasing emission by over 99% (down to ~0.12% of the ammonium nitrogen loading). Without anaerobic digestion, mitigated Nit/DNit decreases the operational carbon footprint with about 80% compared to N/DN. With anaerobic digestion included, about 4 times more carbon is sequestered. In conclusion, the low $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ feature of Nit/DNit no longer offsets its environmental sustainability provided the process is smartly operated.

Keywords: nitrification/denitrification; nitrous oxide; pig slurry; mitigation strategy; carbon footprint

1. Introduction

Anaerobic digestion has been widely used for energy recovery from waste streams (i.e. livestock waste) by producing a renewable energy source, methane. However, the yielding ammonium-rich effluent low in biodegradable organic carbon requires subsequent treatment. The ammonium (NH_4^+) oxidation to nitrite (NO_2^-) and its reduction to nitrogen gas (N_2), termed as nitrification/denitrification (Nit/DNit), is one of the promising technologies for N removal from N-rich waste streams and derivatives, due to economic

incentives. Nit/DNit saves 25% of the total oxygen demand and 40% of the carbon demand, compared to conventional nitrification and denitrification (N/DN) (Fux et al., 2006; Vlaeminck et al., 2012; Lackner et al., 2014; Schaubroeck et al., 2015; Chen et al., 2016).

However, as any biological nitrogen removal process, a potent greenhouse gas nitrous oxide (N_2O) could be emitted from Nit/DNit. N_2O is of significant environmental concern since it not only has approximately 265-fold stronger global warming potential than carbon dioxide (IPCC, 2013), but is also responsible for stratospheric ozone depletion (Ravishankara et al., 2009). Previous studies suggested that N_2O emission could be a significant or even major contributor (up to 80%) to the carbon footprint of wastewater treatment plants (WWTPs) (Joss et al., 2009; Weissenbacher et al., 2010; Wunderlin et al., 2012a; Desloover et al., 2012). During nitrification, two main pathways carried out by ammonia oxidizing bacteria (AOB) contribute to N_2O production: (i) the sequential reductions from NO_2^- to nitric oxide (NO) and to N_2O as the end product, termed as AOB denitrification pathway (Kampschreur et al., 2007; Kim et al., 2010; Yu et al., 2010); (ii) N_2O as a side product during the incomplete oxidation of hydroxylamine (NH_2OH) to NO_2^- , known as NH_2OH oxidation pathway (Chandran et al., 2011; Stein, 2011). Dissolved oxygen (DO), nitrite, ammonia loading rate, pH, alkalinity, etc. were reported to affect N_2O production by AOB (Tallec et al., 2006; Kampschreur et al., 2007; Kim et al., 2010; Law et al., 2011; Wunderlin et al., 2012b; Peng et al., 2014; Peng et al., 2015a; Peng et al., 2015b; Wang et al., 2017). Mathematical modelling and isotopic techniques provided opportunities to further identify the N_2O production pathways by AOB under varying DO and nitrite concentrations (Wunderlin et al., 2013;

Peng et al., 2015; Harris et al., 2015; Peng et al., 2016). Additionally, N_2O is an obligatory intermediate of both denitrification and denitrification with N_2 being the end product for most cases. However, some environmental factors such as DO (Zhu and Chen, 2011; Wunderlin et al., 2012b), carbon source availability (Itokawa et al., 2001; Lu and Chandran, 2010; Quan et al., 2012), free nitrous acid (Zhou et al., 2008), sulfide (Pan et al., 2013), etc. could cause N_2O accumulation during denitrification or denitrification.

The N_2O emission factor, defined as the ratio between N_2O nitrogen emitted and the influent nitrogen loading, displayed a large variation depending on operational conditions and types of processes (Kampschreur et al., 2009; Law et al., 2012). Various levels of N_2O emission were also reported in the partial nitrification process. High concentration of nitrite (500 – 1000 mg N/L) has been shown to exert an inhibitory effect on N_2O production from a nitrification reactor, leading to a substantially lower N_2O emission factor (0.2–1.4%) as compared to those from conventional activated sludge systems (Law et al., 2013). Lv et al. (2016) reported that the N_2O emission factor increased from 1.8% to 2.4% as DO decreased from 0.6 mg O_2 /L to 0.35 mg O_2 /L in a lab-scale nitrification sequencing batch reactor (SBR). In full-scale nitrification reactors, the N_2O emission factors were even higher, ranging from 1.7% to 6.6% (Kampschreur et al., 2008; Desloover et al., 2011; Mampaey et al., 2016).

The lower investment cost and simpler process control drive the implementation of single-stage Nit/DNit, despite of the potential for higher loading in the two-stage approach. However, to date, there is still a lack of research focusing on N_2O production from such a system treating real ammonium-rich wastewater. N_2O emission was negligible (0.07–0.15%) in a pilot-scale continuous stirred tank reactor treating piggery

wastewater (Rajagopal and Béline, 2011). In contrast, Scaglione et al. (2013) measured much higher N_2O emission (3–24%) from a Nit/DNit reactor fed with liquid fraction of digested agro-wastes and found that the ratio between biodegradable organic matter (bCOD) and nitrogen (N) (COD/N) played an important role in determining N_2O production. However, the absence of online and continuous monitoring in these two studies brings difficulties to accurately identify the overall N_2O emission from the reactors. Moreover, effective and efficient mitigation strategies for N_2O emission from side-stream treatment, validated by experimental demonstration, are urgently in need due to the potentially higher N_2O production than that from mainstream processes.

This work aims at a sustainable treatment of high nitrogenous wastewater with minimized N_2O emission. To achieve this, a lab-scale SBR was operated treating the centrate (centrifuge supernatant) from an anaerobic digester processing mainly pig slurry. After obtaining stable performance of nitrification and denitrification via nitrite, N_2O emission from the SBR was on-line monitored under different operational conditions (i.e. DO level, the amount of external carbon, etc.). Based on the observations and data analysis, several mitigation strategies were proposed and compared.

2. Materials and methods

2.1. Set up and operation of the Nit/DNit SBR

A SBR with a working volume of 4.5 L was operated in the laboratory seeded with sludge from a WWTP treating the centrate of pig slurry in Izegem, Belgium. One cycle consisted of anoxic feeding, aerating, anoxic mixing, settling, and decanting. In each 8-hour cycle, the SBR was fed with 0.3 L of the centrate containing 2.0 ± 0.31 g N/L ($n = 9$)

of ammonium from an anaerobic digester processing mainly pig slurry (~84%), supplemented with a co-substrate (~16% Ecofrit). The total Kjeldahl nitrogen (TKN) in the influent was 3.0 ± 0.44 (n = 9) g N/L. The centrate also contained a high level of solids and organic matter (the details refer to Table S1 in Supplementary Material). The volume exchange rate (VER), the hydraulic retention time (HRT) and the targeted nitrogen loading rate were ~6.7%, 5 days and 0.4 g N/L/d, respectively. Glycerin, a by-product of vegetable oil production was supplied at the beginning of the second anoxic phase as external carbon source. The pH in the reactor was controlled in the range of 7.5 – 8.0 by programmed logic controller (PLC) through dosing either 1 M NaHCO_3 or 1M H_2SO_4 . Compressed air was supplied to the reactor during aerobic phases. The DO concentration was controlled in the range of 0 – 1.5 mg O_2 /L with a mass flow controller (0 – 2 L/min STANDARD, Aalborg) and a proportional-integral-derivative controller. The operational temperature of 35 °C was selected to represent the typical temperature of mesophilic digestates. The reactor vessel was jacketed, and the temperature was controlled with a circulating thermostatic water bath (14 L Heated PPO Bath, Thermo Scientific). The solid retention time (SRT) was kept at ~15 days by manually wasting on a daily basis. To monitor the system performance, mixed liquor samples were taken periodically from influent, reactor and effluent for analysis of ammonia nitrogen, TKN, nitrite, nitrate, COD, total suspended solids (TSS), and volatile suspended solid (VSS). After stabilization, characterization of the biomass compositions was conducted using fluorescence in situ hybridization (FISH, the results refer to Supplementary Material).

The reactor was initially loaded at 0.1 g N/L/day. Subsequently the ammonium loading was stepwise increased by shortening the cycle time, while the VER was kept

constant at ~6.7%. Hence, the substrate concentrations would not vary, while the loading rate increased. In particular, due to pulse feeding, the initial high ammonium nitrogen concentration led to a high free ammonia (FA) concentration (around 9 mg NH₃-N/L at pH of 7.8 and temperature of 35 °C), which had a negligible effect on AOB, but rather inhibited nitrite oxidizing bacteria (NOB) (Vadivelu et al., 2007).

2.2. N₂O measuring campaign

To assess the effect of some key parameters on N₂O production, the Nit/DNit SBR was operated under varying DO concentrations and with different amount of external carbon supplement. The effect of nitrite on N₂O production in the system was investigated in one additional set of experiments, where a certain amount of nitrite was dosed into the parent SBR reactor at the beginning of the cycle. Each experiment lasted a minimum of 5 days (one HRT). Mixed liquor samples were taken from the reactor at different phases and from the effluent for NH₄⁺, NO₂⁻, NO₃⁻, COD, TSS, and VSS analysis. The detailed operational conditions are described in Table S2.

The stripped gas from the Nit/DNit reactor was analyzed on-line for N₂O concentration through an Emerson Rosemount X-STREAM Gas Analyzer, preceded by a condenser (4 °C). Data were logged every 5 s. The flow rate of the sampling pump in the analyzer was constant at 100 L/h. The N₂O analyzer was calibrated periodically as per manufacturer's instruction and no signal drift was detected.

A control run was conducted to verify the N₂O production by heterotrophs. Ammonium and nitrite were completely depleted during aerobic phase and anoxic phase, respectively. Subsequently, 75 mg N/L nitrite and 250 mg COD/L glycerin were added in

the beginning of aeration in the next cycle without feeding any centrate. The absence of ammonium substrate would result in a minimum AOB activity. Hence, the on-line measured N_2O emission was mostly attributed to the activity of heterotrophs.

Two sets of short-term experiments (Experiment I and Experiment II) were carried out in the parent SBR reactor to reveal the effect of nitrite and DO levels on N_2O emission rate (defined below). In Experiment I, the N_2O emission was on-line monitored in three consecutive cycles with DO concentrations varying between 0.5 mg O_2/L and 1.0 mg O_2/L . Mixed liquor samples were taken hourly during the 6-hour aerobic phases for analysis of nitrite and nitrate. The DO levels were in the range of 1.4 – 2.8 mg O_2/L during the other three consecutive cycles in Experiment II. A moving window was used to determine each N_2O emission rate and its corresponding nitrite and DO level.

The different evaluated mitigation strategies are described in Figure 1A. In brief, N_2O was on-line monitored in different scenarios with varying DO levels, carbon availability, modes of feeding, aeration, and carbon dosage (continuous or intermittent). The continuous or intermittent modes were achieved by on/off control through a programmed logical controller.

2.3. Calculations

The N_2O emission factor was calculated based on the following equation:

$$N2O_{ef} = \frac{N2O_{em}}{NH_4^+ loading} \times 100 \quad (1)$$

Where $N2O_{ef}$ is the N_2O emission factor, %; $N2O_{em}$ is the mass of N_2O nitrogen emitted over the complete reactor cycle, mg N_2O-N ; $NH_4^+ loading$ is the mass of ammonium nitrogen loading over the reactor cycle, mg NH_4^+-N .

The total N_2O emission factor is the sum of the N_2O emission factor under aerobic phase (the aerobic N_2O emission factor) and N_2O emission factor under anoxic, settling and decanting phases (the anoxic N_2O emission factor), which were calculated based on the following:

$$N2O_{ef}^{ae} = \frac{N2O_{em}^{ae} - N2O_{em}^{ini}}{NH_4^+ \text{ loading}} \times 100 \quad (2)$$

Where $N2O_{ef}^{ae}$ is the aerobic N_2O emission factor, %; $N2O_{em}^{ae}$ is the mass of N_2O nitrogen emitted during the aerobic phase, mg N_2O -N; $N2O_{em}^{ini}$ is the initial peak mass of N_2O emission once aeration starts, caused by stripping of the N_2O accumulated in liquid phase during the non-aerated phases (if applicable), mg N_2O -N.

$$N2O_{ef}^{an} = \frac{N2O_{em}^{an} + N2O_{em}^{ini}}{NH_4^+ \text{ loading}} \times 100 \quad (3)$$

Where $N2O_{ef}^{an}$ is the anoxic N_2O emission factor, %; $N2O_{em}^{an}$ is the mass of N_2O nitrogen emitted during the non-aerated phases, mg N_2O -N.

The stirring of the mixed liquor and the constant gas flow from the reactor headspace into the pump of N_2O analyzer facilitates the mass transfer of N_2O from liquid phase to gas phase, which results in N_2O emission during non-aerating period. The rapid increasing N_2O emission upon the beginning of aeration is also due to mass transfer from aqueous N_2O , accumulated during the anoxic period, to gas phase (if applicable).

The N_2O emission rate is calculated as follows:

$$N2O_{rate} = N2O_{conc} \times Q \quad (4)$$

Where $N2O_{rate}$ is the N_2O emission rate, mg N/h; $N2O_{conc}$ is the N_2O concentration measured in gas phase, mg N/L; Q is the air flow rate in the reactor, L/h.

The ratio between consumed COD and consumed nitrogen was termed COD_{rem}/N_{rem} . The removed nitrogen was calculated as the difference between influent and effluent ammonium nitrogen concentrations. Accurately determining the consumed COD was difficult in this study considering the high COD background levels (refer to Figure 3A). As the parameter is of great practical relevance, its value was estimated based on two assumptions: I) the external carbon from glycerin (added in the anoxic phase) is readily biodegradable, all of which will be consumed anoxically; II) the removed nitrogen during the aerobic phase is due to simultaneous nitrification and denitrification, utilizing internal COD, based on the theoretical COD_{rem}/N_{rem} ratio of 2.3. So the total converted COD is the sum of internal and external COD consumption. Given these assumptions, the obtained values should be interpreted with care.

In addition, to investigate the effect of carbon availability on N_2O production from anoxic denitrification, COD_{an}/N_{an} is defined as the ratio between the mass of COD derived from glycerin dosed in the anoxic phase, and the sum of nitrite and nitrate nitrogen at the beginning of anoxic phase.

2.4. Chemical analysis

NH_4^+ , TKN, TSS, VSS and biochemical oxygen demand (BOD_5) were measured according to standard methods (APHA, 1998). COD was measured by photometric methods using Nanocolor test tubes (Macherey-Nagel, Germany). Nitrite and nitrate were determined on a 761 Compact Ion Chromatograph (Metrohm, Switzerland) equipped with a conductivity detector.

3. Results

3.1. Nit/DNit in SBR

The system performance of the SBR is shown in Figure 2. The details for the operational conditions are shown in Table S2 in Supplementary Information. It should be noted over days 89 – 108, the DO level was elevated to 2.2 mg O₂/L to evaluate its impact on 1) NOB suppression, due to the fact that *Nitrospira* is preferably out-selected at high DO, while *Nitrobacter* is preferably suppressed at low DO (Regmi et al., 2014; Blackburne et al., 2008); 2) nitrite reduction, with varying external COD dosage; 3) N₂O emission, since DO level has been shown as an important factor shifting N₂O pathways and thus leading to varying N₂O emissions (Peng et al., 2014). Within the first 30 days, ammonium loading rate increased from ~0.1 g N/L/day to ~0.4 g N/L/day, while the corresponding cycle time decreased from 32 hours to 8 hours (Figure 2A). Aeration failure led to incomplete ammonium oxidation on day 35. Hence, the ammonium loading rate was lowered to help the microbial activity to recover. The reactor was running with full capacity since day 60 at a NH₄⁺ loading rate in the range of 0.3 – 0.4 g N/L/day. TKN loading rate displayed a similar trend and was stable in the range of 0.4 – 0.55 g N/L/day. The removal efficiencies for ammonium and TKN were almost 100% and 80%, respectively for most of the testing period (Figure 2A). At steady state, ~40% of nitrogen was removed during aerobic phase. Based on the assumption above, the COD_{rem}/N_{rem} is estimated at 2.8 with ~33% of internal and ~67% of external carbon consumed by heterotrophic denitrifiers.

With some seldom exceptions, the effluent ammonium, nitrite and nitrate were depleted shortly after the start-up (Figure 2B). As mentioned above, there was ammonium accumulation up to ~770 mg N/L in the effluent due to aeration failure. The

subsequent nitrite accumulation up to ~78 mg N/L was due to the fact that the high FA concentration negatively affected the activity of AOB, NOB and denitrifiers. During Period II, III and IV from day 89 to day 108 (Table S2), the average DO level in the Nit/DNit reactor was changed from ~0.85 mg O₂/L to ~2.2 mg O₂/L to evaluate N₂O response under varying conditions (refer to Figure 4). The buildup of nitrite and nitrate in the effluent during these periods suggested that the higher DO concentrations recovered NOB activity (Figure 2B). Given the insufficient carbon ($\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ around 2.8) for complete denitrification, nitrate and nitrite started to accumulate in the effluent. The decrease of DO to 0.5 mg O₂/L in the following period (Period V, Table S2) successfully suppressed the NOB activity again. Throughout the testing period, effluent concentrations of NH₄⁺, NO₂⁻ and NO₃⁻ were mostly below 20 mg N/L, indicating a good system performance (Figure 2B). The TKN concentrations in the effluent varied between 430 mg N/L and 810 mg N/L during the 160 days (Figure 2B).

The nitrate and nitrite levels at the end of the aerobic phase were used as indicators for nitrate formation and nitrite removal (Figure 2C). At the beginning, nitrite and nitrate concentrations were around 170 mg N/L and 80 mg N/L, respectively, both of which decreased rapidly within the first five days. In the 160-day reactor operation, the nitrate was kept at a low level, indicating a good performance of nitrification. Concomitantly, the nitrite profile displayed a decreasing trend, indicating an improving performance of denitrification under aerobic conditions. At the higher DO concentrations from day 89 to day 108 (Period II, II and IV, Table S2), nitrate became the dominant nitrogen substrate over nitrite, indicating a transformation from the nitrification to complete nitrification. By lowering the DO set point, the Nit/DNit system was back to normal.

Figure 2D shows the solid content in the SBR. At the beginning, the reactor contained ~ 3 g VSS/L, followed by a rapid increase up to ~14 g VSS/L due to the high level of solids in the influent as shown in Table S1. The effluent VSS changed correspondingly. After reaching the steady state, the VSS concentrations in the reactor and in the effluent were in the ranges of 10 g/L – 14 g/L and 7 – 10 g/L, respectively. The VSS/TSS was relatively stable, which was between 0.5 and 0.6 over the test period. Due to the high solids concentration in the pig slurry, the VSS concentration in the SBR (10-14 g/L) was much higher than the typical VSS concentration in activated sludge plants (3-5 g/L). SRT was controlled at a constant level (around 15 days) by daily manual sludge wasting, taking the effluent loss of suspended solids into consideration. Despite of the high solids concentration in the influent, washout of AOB activity was not observed during operation, and the system performance performed at satisfactory removal rate, efficiency and stability, as shown in Figure 2.

3.2. N₂O emission from baseline operation

As an example, Figure 3 presents gas-phase N₂O concentrations along with N, DO, pH, and COD in one SBR cycle under baseline conditions (Table S2). Similar trends were observed in the profiles of other cycles with the same operational conditions. Time 0 corresponded to the beginning of the aerobic phase. The total COD (tCOD) and soluble COD (sCOD) were ~24.5 g COD/L and ~5.5 g COD/L, respectively (Figure 3A). Due to the high background COD concentrations in the reactor, the consumed COD by aerobic growth of heterotrophs and anoxic growth of denitrifiers could not be accurately determined by the photometric method. The initial ~120 mg N/L of NH₄⁺ was depleted

within 6 hours (the end of the aerobic phase), resulting in an ammonia oxidation rate of ~19.4 mg N/L/hour (Figure 3B). The NO_2^- concentration kept increasing over time, peaked at ~57 mg N/L during the aerobic phase and was completely denitrated during the anoxic phase. NO_3^- was below 2 mg N/L during the entire cycle time. DO varied between 0 and 1.5 mg O_2 /L, while pH was around 7.7 for most of the time, but rapidly increased to ~7.9 after feeding of centrate (Figure 3C). N_2O concentration in ppm_v was simultaneously monitored by the gas analyzer under the conditions of 1 atm and 22 °C. As shown in Figure 3D, N_2O gradually increased from 0 to ~150 ppm_v during the aerobic phase and further elevated up to 280 ppm_v during the anoxic phase. The N_2O emission factor was ~15.6% for the whole cycle and the aerobic N_2O emission factor was around 9.5%.

3.3. N_2O emission corresponding to varying NO_2^- , DO, and $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$ levels

The N_2O emission factors obtained from daily analysis of baseline and Period I – V (Table S2) were correlated to nitrite accumulation (Figure 4). When the reactor was operated at DO concentrations below 1.5 mg O_2 /L (Figure 4A), both the total and aerobic N_2O emission factors were linearly dependent on nitrite accumulation ($R^2 = 0.76$). However, at DO levels above 1.5 mg O_2 /L (Figure 4B), the increase of nitrite concentration from ~33 mg N/L to ~188 mg N/L had negligible impact on the total and aerobic N_2O emission factors. The corresponding R^2 was only 0.3. With similar nitrite concentration, lower N_2O emission was observed at high DO levels (Figure 4B) as compared to lower levels (Figure 4A). At a low DO concentration, around 81% of N_2O was emitted during the aerobic phase (Figure 3). However, at a high DO level only ~11%

of the measured N_2O was emitted during the aerobic phase, which suggests incomplete denitrification as dominant mechanism of N_2O production (Figure 4B).

N_2O emission rates from two sets of short-term experiments (Experiment I and Experiment II, as described above) were plotted against varying nitrite accumulations and DO set points in Figure S1. The results further confirmed the strong dependency of N_2O emission rate on nitrite accumulation ($R^2 = 0.93$) at low DO levels (0 - 1.0 mg O_2/L) and on DO concentration ($R^2 = 0.93$) at high DO levels (1.4 - 2.8 mg O_2/L).

As shown in Figure S2 the anoxic emission factor was strongly dependent on $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$. As $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$ increased from ~1.4 to ~14, anoxic N_2O emission factor decreased substantially. The highest anoxic emission factor of ~21% occurred at $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$ of ~1.9, whilst the lowest (~0.41%) was observed at the highest $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$ of ~14.

4. Discussion

4.1. Single-stage Nit/DNit process can be a significant contributor to N_2O emission during wastewater treatment

In this study, the single-stage Nit/DNit process was successfully implemented in a bench-scale SBR treating centrate from pig slurry co-digestion. Suppression of NOB was achieved using a combination of low dissolved oxygen levels (average 0.85 mg O_2/L), high temperature (35°C), and relatively high initial FA concentration (around 9 mg $\text{NH}_3\text{-N/L}$). After stabilization, the system was able to remove approximately 100% of ammonium (~2 g N/L in the influent) and 80% of TKN (~3 g N/L in the influent) with minimum nitrite and nitrate in the effluent, at a reasonably high nitrogen loading rate of

346 ~0.4 g N/L/d.

347 This work fills the gaps with regard to accurate identification of N₂O emission from
348 the single-stage Nit/DNit system. Under baseline operational conditions, about 17% of
349 ammonium nitrogen load was emitted as N₂O gas from the Nit/DNit SBR, which could
350 be a significant source of carbon footprint if implemented in full scale (Rodriguez-
351 Caballero et al., 2015). This value is consistent with the reported emission levels on
352 similar waste streams. Scaglione et al. (2013) reported N₂O emission factors of 2–20% in
353 off-gas samples taken from a pilot Nit/DNit SBR treating the liquid fraction of agro-
354 digestate.

355 However, N₂O emission from our system is substantially higher than the reported
356 values (0.2–2.4%) in lab-scale partial nitrification SBRs fed with synthetic wastewater in
357 the absence of COD, which may be attributed to three reasons (Law et al., 2013; Lv et al.,
358 2016). First of all, heterotrophic denitrification may contribute to N₂O production during
359 anoxic phase. Further data analysis indicated that ~26% of the total N₂O emission was
360 from anoxic denitrification during baseline operation. Secondly, heterotrophic denitrification
361 may also contribute to N₂O production during the aerobic phase. According to the mass
362 balance, about 40% of influent ammonium ended as N₂ gas, demonstrating the
363 simultaneous nitrification and denitrification in the presence of oxygen. The denitrified
364 nitrogen had the opportunity to be transformed to N₂O gas. To identify the contribution of
365 aerobic denitrification to N₂O production, an additional test was performed in SBR
366 mimicking the conditions of aerobic phase in baseline operation, but in the absence of
367 ammonium and with initial addition of nitrite and glycerin. As shown in Figure S3, the
368 DO level in the reactor was controlled between 0 – 1.0 mg O₂/L. The nitrite level was

initially around 75 mg N/L and decreased gradually to 34 mg N/L at the end of 5-h aerating period. On the other hand, N₂O concentration mostly fluctuated between 2.5 ppm_v and 10 ppm_v, suggesting a minimum contribution by aerobic heterotrophic denitrification (~6% of the total aerobically emitted N₂O under baseline conditions).

Finally, the varying nitrite levels would exert diverse or even opposite impacts on N₂O production. Law et al. (2013) found that high nitrite accumulation (500 – 1000 mg N/L) in partial nitrification SBR could lower N₂O production via AOB denitrification pathway. In our reactor, the build up of nitrite was within the range of 30 – 60 mg N/L, which was far below the inhibition threshold (Law et al., 2013). Moreover, the relatively high pH (averaged at 7.7) also eliminated the possibility of free nitrous acid inhibition. In essence, the moderate nitrite accumulation may play a key role in triggering N₂O production by AOB. Peng et al. (2015a) revealed that N₂O emission factor increased from ~6% to 13% as nitrite concentration increased from 3 mg N/L to 50 mg N/L at DO level of 0.85 mg O₂/L in a enriched nitrifying culture.

4.2. Metabolic pathways of N₂O production during nitrification and denitrification

As shown in Figure 4A, N₂O emission factor increased with the increase of nitrite at DO concentrations below 1.5 mg O₂/L. AOB denitrification pathway was activated under oxygen limiting condition and promoted by nitrite accumulation (Tallec et al., 2006; Kampschreur et al., 2007; Wunderlin et al., 2012b; Peng et al., 2014). The stimulated N₂O emission at higher nitrite level was possibly related to a promoted expression of nirK gene or increased activity of NO₂⁻ reductase with increasing substrate concentration (Beaumont et al., 2004). Thus, at lower DO (<1.5 mg O₂/L), AOB denitrification was

possibly the major contributor to N₂O production by nitrification.

However, both AOB denitrification and NH₂OH oxidation pathways made contribution to N₂O emission from the Nit/DNit system at higher DO levels. On one hand, the N₂O emission factors were not correlated to nitrite concentrations any more at DO above 1.5 mg O₂/L (Figure 4B). The increase of DO concentrations completely altered the dependency of N₂O production on nitrite accumulation. It is likely that the higher DO triggered the occurrence of NH₂OH oxidation pathway since the observation here was in line with the prediction by a two-pathway N₂O model showing that the increase of nitrite had no effect on N₂O production via NH₂OH oxidation pathway (Peng et al., 2015a). Ni et al. (2014) and Chandran et al. (2011) also reported that an elevated DO concentration favored NH₂OH oxidation pathway. On the other hand, at similar nitrite conditions, a lower N₂O emission factor was observed at high DO levels (>1.5 mg O₂/L) compared to that at lower DO levels (<1.5 mg O₂/L) (Figure 4). This observation was further validated by the results from two additional experiments, where the N₂O emission rates were correlated to varying nitrite and DO levels (Figure S1). An elevated DO level decreased the importance of AOB denitrification pathway in both mixed culture (Peng et al., 2015a) and pure AOB cultures (Poth and Focht, 1985; Remde and Conrad, 1990). Hence, these observations together confirmed that N₂O emission at higher DO (>1.5 mg O₂/L) resulted from a combination of AOB denitrification pathway and NH₂OH oxidation pathway. The two important compounds, oxygen and nitrite, competed for electrons generated by NH₂OH oxidation, leading to the varying contributions of the two known pathways (Anderson et al., 1993; Peng et al., 2015a).

N₂O production by nitrification served to be the major contributor in this Nit/DNit

system in comparison to N_2O production by denitrification. The contribution of nitrification including both AOB denitrification and NH_2OH oxidation pathways accounted for ~68% of total N_2O emission under baseline conditions. In contrast, with aid of isotopic techniques and denitrification functional gene transcriptome analysis, Ishii et al. (2014) identified that heterotrophic denitrification was the main contributor to N_2O production in a partial nitrification aerobic granule reactor, fed with ammonium and acetate. The divergence is possibly due to the fact that the granules create extensive anoxic layers, where denitrification occur in the absence of oxygen, as opposed to the mild gradients expected in the flocculent sludge used in this study. With the addition of nitrite (Period I in Table S2), it was seen that the total, aerobic and anoxic N_2O emission factors in the Nit/DNit reactor were all substantially promoted (Figure 4A). As nitrite increased from ~20 to ~130 mg N/L, the contribution of anoxic denitrification to the total N_2O emission increased from ~10% to ~53%, accompanied by a decrease of the contribution of aerobic nitrification (Figure 4A). The increased build up of nitrite led to a lower $\text{COD}_{\text{an}}/\text{N}_{\text{an}}$ ratio and the resulting carbon limitation yielded higher N_2O production from denitrification (Itokawa et al., 2001; Lu and Chandran, 2010; Scaglione et al., 2013).

4.3. Minimizing N_2O from the Nit/DNit reactor

Animal waste, the biggest wasted nitrogen mass flow through our economies, was used as a model in this work (Coppens et al., 2016). Comparing to some full-scale partial nitrification reactors (Kampschreur et al., 2008; Desloover et al., 2011; Mampaey et al., 2016), the Nit/DNit SBR in this work generated much more N_2O (~17%) in baseline operation (Figure 1B). The nitrite produced by AOB will be simultaneously consumed by

denitrifiers at aerobic phase in the Nit/DNit system. A much lower nitrite (30 – 60 mg N/L) than that in partial nitrification leads to N₂O spikes, which has been also found in lab-scale (Peng et al., 2015a) and full-scale studies (Kampschreur et al., 2009). Consequently, further minimizing the nitrite accumulation would be an effective way to limit N₂O production.

Based on previous observations, high DO concentration was able to reduce aerobic N₂O emission (Figure 4) and high glycerin supplement led to lower anoxic N₂O emission (Figure S2). By combining supplement of sufficient carbon (COD_{rem}/N_{rem} of 6.5) and application of elevated DO level (averaged at ~2.2 mg O₂/L) in Strategy I, the N₂O emission factor was down to ~2.2% (Figure 1B). However, these implemented conditions reactivated NOB (Figure 2) and thus lost the cost savings brought by the Nit/DNit system concerning the lower oxygen and carbon demand. Additionally, more energy would be consumed to maintain DO at high levels and carbon was excessively wasted in this scenario. Hence, the subsequent scenarios were all investigated at low DO levels (averaged at 0.35 mg O₂/L) and low COD_{rem}/N_{rem} ratio (around 2.8).

Different aeration patterns were previously reported to deliver varying N₂O dynamics in WWTPs (Rodriguez-Caballero et al., 2015). N₂O emission was reduced by replacing continuous aeration with intermittent aeration in lab-scale reactors treating swine wastewater (Osada et al., 1995; Béline and Martinez, 2002). Implementation of short aeration periods during intermittent aeration was found to significantly minimize N₂O emission in both lab-scale (Béline and Martinez, 2002) and full-scale investigations (Rodriguez-Caballero et al., 2015). In Strategy II, we applied intermittent aeration instead of continuous aeration during aerobic phase with aeration to non-aeration ratio ranging

from 0.43 to 2.33. The overall N_2O emission ($\sim 11\%$) was reduced by $\sim 35\%$ compared to baseline operation ($\sim 17\%$) (Figure 1B) and the varying lengths of aerating period showed a negligible impact on N_2O emission. The carbon loss during aerating period may cause carbon limitation during non-aeration, resulting in a nitrite build-up of ~ 40 mg N/L in Strategy II. To further utilize the bCOD in the wastewater, anoxic intermittent feeding (Strategy III) was implemented, leading to a decreased N_2O emission by 20% compared to Strategy II (Figure 1B).

The optimal scenario with minimum N_2O emission factor of 0.12% was obtained by applying intermittent patterns of aeration, anoxic feeding and anoxic carbon dosing in Strategy IV. The consistently changing trend of nitrite accumulation against N_2O emission factor in Figure 1B demonstrated that the minimized N_2O was attributed to promoted consumption of nitrite or even N_2O itself by heterotrophic denitrifiers. Figure S4 presents the liquid-phase N and DO along with gas-phase N_2O in the Nit/DNit SBR with Strategy IV applied. The concentrations of NH_4^+ , NO_2^- and NO_3^- were all below 1 mg N/L during the entire cycle. The intermittent aeration created alternate 7-min aerobic phase and 3-min anoxic phase. The N_2O peak of ~ 6 ppm_v occurred upon the start of the aeration. We also identified the optimal $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ as 2.8 in Strategy IV. The lower $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ ratio (2.5) substantially triggered N_2O emission ($\sim 2.6\%$), while higher $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ of 3.6 (a cost factor) slightly reduced N_2O emission ($\sim 0.054\%$) (Figure 1B).

In partial nitrification/anammox, several operational conditions are similar to nitritation/denitritation, and both processes strive for NOB suppression and minimum N_2O emission. A study by Wang et al. (2016) revealed that sludge treatment based on free nitrous acid (FNA) exposure can mitigate N_2O emission from a partial nitritation

system. Hence, future work may focus on combining optimal aeration and feeding regimes with FNA-based sludge treatment, and reinforce mitigation of N_2O emissions in a range of shortcut nitrogen removal applications.

4.4 Impact extrapolation

Animal slurry processing can avoid severe environmental problems related to intensive livestock production to a considerable extent, yet comes at a cost, and should not generate high alternative emissions (Pintucci et al., 2016). Based on a desktop extrapolation study, selected impact categories of direct economic and environmental relevance were evaluated: (i) key operational cost categories and (ii) carbon gas footprint, based on direct and indirect sources and sinks. The considered pig slurry treatment lines consisted of a centrifuge (solid/liquid separation) followed by a biological nitrogen removal stage. Furthermore, the effect of an anaerobic (co-) digestion stage prior to the centrifuge was evaluated. Nitrogen treatment scenarios compared the baseline scenario of N/DN, at a typical N_2O emission (0.75% of the TN loading) (Lemmens, et al. 2007) and with external methanol addition (fossil C source), to the Nit/DNit process, at worst and best N_2O emission (11-0.080% of the N loading) and, if necessary, with external glycerol addition (biogenic C-source). Further calculation assumptions are detailed in Tables S3&S4.

Table S3 displays the operational cost differences of the scenarios. Applying the Nit/DNit process lowers the requirements for external carbon and aeration so that the operational cost falls by 1.07-2.07 EUR/m³ of centrate, and hence 0.952-1.84 EUR/m³ of raw slurry. For the treatment line without digestion, a global processing rate of about 15

EUR/m³ can be assumed (Lemmens, et al. 2007). The Nit/DNit scenario hence yields an overall saving potential of about 12% of the total treatment cost.

The operational carbon footprint of the full treatment lines is compared in Table S4. And the key findings are highlighted in Figure 5. To exemplify the potential negative impact of improper Nit/DNit operation, a scenario with the highest measured N₂O emission (11.3% of total N loading) was included in the comparison. In the absence of anaerobic digestion (AD), this worst case Nit/DNit (Baseline, Figure 1A) increases the carbon footprint with about a factor 8, while the mitigated scenario (Strategy IV, Figure 1A) can lower the footprint with 83%, both compared to the N/DN baseline scenario (Figure 5). Including AD, the net carbon sequestration in the mitigated Nit/DNit scenario is approximately 4 times better than in the N/DN process (Figure 5). The high N₂O emission Nit/DNit scenario, in contrast, strongly outweighs the carbon sequestration potential of AD. The typical carbon footprint from animal slurry management is 35 - 132 kg CO₂/m³ (including collection, transportation, storage and land application), depending on the practice and farm size (Aguirre-Villegas and Larson, 2016). Maloperation of a nitrogen removal stage can strongly aggravate this, as shown from the worst-case Nit/DNit scenarios (102 - 187 kg CO₂/m³ of raw slurry, derived from 115 - 210 kg CO₂/m³ centrate). The slurry processing by anaerobic digestion and Nit/DNit on the other hand could allow for a considerable overall reduction of 17-64% (best case; -22.5 kg CO₂/m³ of slurry, from -25.3 kg CO₂/m³ of centrate).

Production of renewable energy is one of the objectives of including AD in a treatment line for animal waste slurry. To have a sustainable environmental impact, the carbon footprint of such installation should be below the one of current electricity

generation, i.e. 0.276 kg CO₂ emitted/kWh_{el} produced (EU-28 in 2014). However, the operational carbon footprint of the improper Nit/DNit operation (worst case) yields a CO₂ footprint that is more than 5 times higher. In order to restrict this impact to 10-50% of the one of conventional electricity generation, the maximum allowed N₂O emissions for Nit/DNit and N/DN are respectively 2.3-3.2% and 1.5-2.3% of the TN loading. The operational footprint of the best-case Nit/DNit AD scenario (0.01 kg CO₂/kWh_{el}) illustrates that proper nitrogen removal can enable a sustainably sound solution in comparison to the lowest footprint of other renewable power sources like wind (0.01-0.12 kg CO₂/kWh_{el}) and photovoltaics (0.05-0.25 kg CO₂/kWh_{el}) (Bhat and Prakash, 2009).

Retrofitting an existing N/DN system to a Nit/DNit process is fairly simple, including adjustment of a periodic feeding strategy, alternate aerobic and anoxic periods, DO control at relatively low setpoint and a periodic dosage of external carbon (no need for slurry, only for digestate). We believe that our findings on single-stage Nit/DNit systems treating co-digested pig slurry centrate could be extrapolated to the treatment of other high nitrogenous wastewater (i.e. landfill leachates, industrial effluent, etc.) and potentially serves as a guideline for operation aiming at minimum carbon footprint.

5. Conclusion

A SBR reactor performing Nit/DNit was operated to treat centrate of pig slurry co-digestion with a nitrogen loading rate of ~0.4 g N/L/d. Approximately 100% of ammonium and 80% of TKN were removed with minimum effluent nitrite and nitrate. Under baseline conditions, around 17% of the nitrogen loading was emitted as N₂O. The aerobic nitrification and anoxic denitrification contributed to ~68% and ~26%, respectively

with the remaining from aerobic denitrification. At low DO concentration (<1.5 mg O_2/L), N_2O emission was linearly dependent on nitrite accumulation, suggesting that AOB denitrification pathway dominated. At elevated DO level (>1.5 mg O_2/L), nitrite had negligible effect on N_2O production, while further increase of DO concentration inhibited total N_2O emission, indicating the coexistence of AOB denitrification and NH_2OH oxidation pathways. Effective N_2O mitigation was achieved by combining the intermittent patterns of aeration, anoxic feeding, and anoxic carbon dosage, where the N_2O emission factor was significantly reduced to $\sim 0.12\%$. Based on desktop extrapolation study, Nit/DNit, compared to conventional nitrification/denitrification, can lower the operational costs with around 1.0-1.8 EUR/ m^3 . Without anaerobic digestion, mitigated Nit/DNit decreases the operational carbon footprint with about 80% compared to N/DN. With anaerobic digestion included, about 4 times more carbon is sequestered. Our findings have important implications for sustainable treatment of high nitrogenous wastewater through smart operation.

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Figure 1. The impact of different mitigation strategies on total N_2O emission from the SBR. (A: Schematic representation of different mitigation experiments; B: N_2O emission from different mitigation strategies, Strategy IV was investigated under three $\text{COD}_{\text{rem}}/\text{N}_{\text{rem}}$ levels as shown above.).

Figure 2. The system performance of Nit/DNitr SBR treating manure centrate, operated with DO range of 0 - 1.5 mg O_2 /L and pH range of 7.5 - 8.0. (A: loading rates and removal efficiencies of NH_4^+ and TKN; B: effluent TKN, NH_4^+ , NO_2^- and NO_3^- ; C: NO_2^- and NO_3^- accumulations at the end of aerobic phase; D: solid content in the reactor and the effluent).

Figure 3. Profiles of (A) tCOD and sCOD; (B) NH_4^+ , NO_2^- and NO_3^- ; (C) DO and pH along with (D) gaseous N_2O in one SBR cycle under baseline conditions.

Figure 4. Relationship between N_2O emission factor (total and aerobic) and nitrite accumulation at varying DO concentrations (A: DO levels below $1.5 \text{ mg O}_2/\text{L}$; B: DO levels above $1.5 \text{ mg O}_2/\text{L}$).

Figure 5. Extrapolated operational carbon footprint of pig slurry processing lines (without or with anaerobic digestion, AD): Comparison between nitrification/denitrification (N/DN) and nitritation/denitrification (Nit/DNIt), expressed per m³ of slurry treated, in the respective emission scenarios of 0.0075 and 0.0008 kg N₂O-N/kg N loaded.

Highlights

- Nit/DNit can lower operational costs with around 1-1.8 EUR/m³, compared to N/DN.
- Improper Nit/DNit operation gave a very high N₂O emission: 17% of the NH₄⁺-N load.
- Nitritation was the major N₂O source, stimulated by high nitrite and low DO levels.
- Synergy between aeration, feeding and carbon dosage mitigated the emission by 99%.
- The low COD/N requirement for Nit/DNit no longer offsets ecological sustainability.