



Nitrite build-up effect on nitrous oxide emissions in a laboratory-scale anaerobic/aerobic/anoxic/aerobic sequencing batch reactor

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ABSTRACT

Biological wastewater treatment processes with biological nitrogen removal are potential sources of nitrous oxide (N₂O) emissions. It is important to expand knowledge on the controlling factors associated with N₂O production, in order to propose emission mitigation strategies. This study therefore sought to identify the parameters that favor nitrite (NO₂⁻) accumulation and its influence on N₂O production and emission in an anaerobic/aerobic/anoxic/aerobic sequencing batch reactor with biological nitrogen removal. Even with controlled dissolved oxygen concentrations and oxidation reduction potential, the first aerobic phase promoted only partial nitrification, resulting in NO₂⁻ build-up (ranging from 29 to 57%) and consequent N₂O generation. The NO₂⁻ was not fully consumed in the subsequent anoxic phase, leading to even greater N₂O production through partial denitrification. A direct relationship was observed between NO₂⁻ accumulation in these phases and N₂O production. In the first aerobic phase, the N₂O/NO₂⁻ ratio varied between 0.5 to 8.5%, while in the anoxic one values ranged between 8.3 and 22.7%. Higher N₂O production was therefore noted during the anoxic phase compared to the first aerobic phase. As a result, the highest N₂O fluxes occurred in the second aerobic phase, ranging from 706 to 2416 mg N m⁻² h⁻¹, as soon as aeration was triggered. Complete nitrification and denitrification promotion in this system was proven to be the key factor to avoid NO₂⁻ build-up and, consequently, N₂O emissions.

Keywords: nitrite accumulation, nitrous oxide production and emission, sequencing batch reactor.

Efeito do acúmulo de nitrito na emissão de óxido nitroso de um reator em batelada sequencial anaeróbico/aeróbico/anóxico/aeróbico em escala de laboratório

RESUMO

Os processos de tratamento biológico de esgotos com remoção biológica de nitrogênio são potenciais fontes de emissão de óxido nitroso (N₂O). No entanto, é importante ampliar o conhecimento dos principais fatores de controle associados à produção de N₂O para propor estratégias de mitigação de sua emissão. O objetivo deste estudo foi identificar os parâmetros



que favoreceram o acúmulo de nitrito (NO_2^-) e sua influência na produção e emissão de N_2O em um reator em batelada sequencial anaeróbio/aeróbio/anóxico/aeróbio com remoção de nitrogênio. Mesmo com a concentração de oxigênio dissolvido e o potencial redox controlados, a primeira fase aeróbia promoveu apenas a nitrificação parcial resultando em acúmulo de NO_2^- (variando de 29 a 57%) e geração de N_2O . Este NO_2^- não foi totalmente consumido na fase anóxica subsequente promovendo uma produção ainda maior de N_2O pela desnitrificação parcial. Foi observada uma relação direta entre o acúmulo de NO_2^- nessas fases e a produção de N_2O . Enquanto na primeira fase aeróbia a razão $\text{N}_2\text{O}/\text{NO}_2^-$ variou entre 0,5 a 8,5%, na anóxica foi entre 8,3 e 22,7%. Portanto, houve uma maior produção de N_2O durante a fase anóxica do que na primeira fase aeróbia. Com isso, os maiores fluxos de N_2O ocorreram na segunda fase aeróbia, variando de 706 a 2416 $\text{mg N m}^{-2} \text{h}^{-1}$, assim que a aeração foi acionada. A promoção da nitrificação e da desnitrificação completas neste sistema mostrou ser o fator chave para evitar o acúmulo de NO_2^- e, conseqüentemente, a emissão de N_2O .

Palavras-chave: acúmulo de nitrito, produção e emissão de óxido nitroso, reator em batelada sequencial.

1. INTRODUCTION

High nitrogen (N) concentrations in effluents may cause eutrophication and deterioration of recipient water bodies. N overloads favor microalgae and water plant growth, which may release toxins into the water (von Sperling, 2005). Although non-toxic, geosmin and 2-methylisoborneol (2-MIB), two products released by cyanobacteria, can influence drinking water organoleptic characteristics, representing an obstacle to water treatment (Freitas *et al.*, 2008). In order to prevent eutrophication, wastewater treatment plants (WWTPs) must be improved to ensure that N loads to receiving water bodies are within the limits stipulated by local legislation (Yang *et al.*, 2017).

An economically viable and widely studied alternative for N removal is the application of biological processes involving nitrification and denitrification (von Sperling, 2005). However, the possibility of N_2O release exists in both reactions, thus resulting in an anthropogenic source of this gas into the atmosphere (Wrage *et al.*, 2001). In the troposphere, N_2O is a chemically stable and long-lived greenhouse gas, with a global warming potential about 265 times that of carbon dioxide (CO_2) (IPCC, 2014). Furthermore, in the stratosphere, N_2O is the most emitted gas from anthropogenic sources displaying ozone (O_3) depletion potential (Ravishankara *et al.*, 2009). The highest N_2O emission rates in WWTPs occur on those that apply biological processes, especially those that operate nitrification and denitrification processes in activated sludge (IPCC, 2019).

During nitrification under aerobic conditions, the ammonium ion (NH_4^+) is converted to hydroxylamine (NH_2OH), which is, in turn, oxidized to nitrite (NO_2^-) with the participation of ammonia-oxidizing bacteria (AOB) under alkaline conditions, while NO_2^- is oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). During denitrification under anoxic conditions, facultative heterotrophic bacteria convert NO_3^- into molecular N (N_2) (Wrage *et al.*, 2001). N_2O production is commonly attributed to three pathways: (1) partial nitrification, as a by-product of NH_2OH oxidation; (2) nitrifier denitrification, which can occur under oxygen-limiting conditions, as an intermediate product; and (3) heterotrophic denitrification, where N_2O is an intermediate product but can be released when the process is incomplete (Duan *et al.*, 2017; Terada *et al.*, 2017). Variations in N_2O production and emissions occur according to the type of applied treatment process and configuration and operational parameters (Law *et al.*, 2012).

N_2O generation is usually associated with dissolved oxygen (DO) concentrations, NH_4^+ and NO_2^- accumulation, pH and organic carbon availability (Duan *et al.*, 2017; Vasilaki *et al.*,

2019). Pijuan *et al.* (2014) reported N_2O emissions almost ten-fold higher when altering a pilot plant system from continuous operation to a sequencing batch reactor (SBR). The authors attributed the N_2O increases to the transient conditions between the anoxic and aerobic stages. Rodriguez-Caballero *et al.* (2015) also reported higher emissions in a full-scale SBR due to anoxic/aerobic transition. The authors also pointed out NO_2^- build-up and the length of the aeration phases as contributors. Thus, SBR may become a potential source of emissions when applying operational conditions that favor N_2O generation.

Knowledge of parameters that affect N_2O production during the nitrification and denitrification stages is necessary to improve the sustainability of the process (Blum *et al.* 2018). Therefore, this study evaluated and identified the parameters responsible for NO_2^- build-up and its effects on N_2O production and emission in an SBR operated under anaerobic/aerobic/anoxic/aerobic conditions.

2. MATERIAL AND METHODS

2.1. SBR operation

The study was carried out in a laboratory-scale anaerobic/aerobic/anoxic/aerobic SBR (Figure 1A). The different SBR system phases were adjusted and regulated by a programmable logic controller (PLC), favoring higher DO and oxidation reduction potential (ORP) control. The reactor comprises 8.1 L of working volume and treats 4 L during each 8-hour cycle (Figure 1B). An air compressor pump was used to provide system aeration, with an air flow rate of 120 L h^{-1} . Peristaltic pumps were used for the feeding and discharge of raw and treated wastewater, respectively. A mixed liquor volume was removed from the reactor during each cycle by a peristaltic pump, to guarantee a solid retention time (SRT) of 30 days.

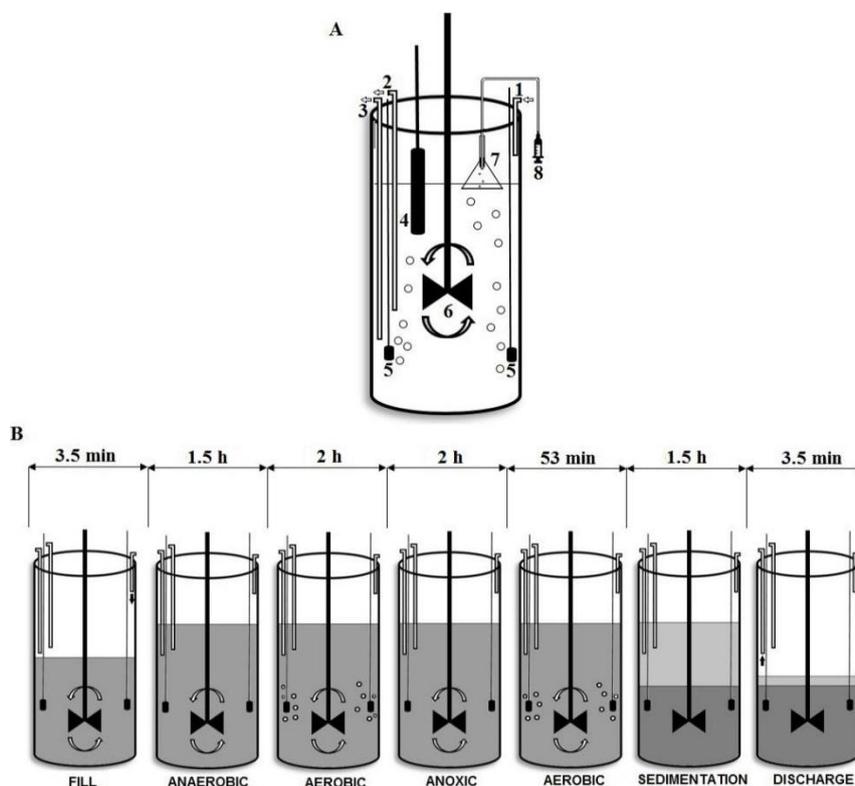


Figure 1. (A) SBR schematic: 1 - wastewater inlet; 2 - mixed liquor outlet; 3 - treated wastewater outlet; 4 - DO, pH and ORP probe; 5 - oxygen diffuser; 6 - mechanical agitator; 7 - inverted funnel; 8 - syringe. (B) SBR cycle schematic.

The reactor was inoculated with sludge from a WWTP designed to treat sanitary wastewater from a 2,500 population equivalent (PE) and fed with synthetic wastewater, which was prepared by adapting the formulation used by Holler and Trösh (2001). The synthetic wastewater was composed of casein peptone (500 mg N L⁻¹), beef extract (323 mg N L⁻¹), dibasic potassium phosphate (35 mg N L⁻¹), sodium chloride (24 mg N L⁻¹), urea (23 mg N L⁻¹), calcium chloride dihydrate (23 mg N L⁻¹) and magnesium sulfate heptahydrate (11 mg N L⁻¹). SBR stabilization took 2 months. After this period, samples were collected for efficiency and N₂O production and emission assessments.

2.2. Sampling and analysis

Monitoring took place for six consecutive weeks, where one sampling of one cycle was performed (one cycle per week). Throughout the sampling stage, raw and treated wastewaters were collected for chemical oxygen demand (COD), dissolved organic carbon (DOC) and total nitrogen (TN) analyses. In addition, volatile suspended solids (VSS) were analyzed in the discharged mixed liquor. After each metabolic phase, mixed liquor samples were collected for NO₂⁻ and NO₃⁻ analysis. Dissolved and emitted N₂O sampling were also carried out during this period.

COD and VSS were determined according to APHA *et al.* (2012). DOC and TN analyses were performed on a TOC-L and TN Analyzer model TOC-L/TNM-L (Shimadzu). NO₂⁻ and NO₃⁻ analyses were performed by a Personal IC ion chromatograph (Metrohm) with conductivity detector, using a Polyvinyl alcohol with quaternary ammonium groups column (Metrosep A Supp 5 – 150x4.0 mm); a solution of Sodium Carbonate (3.2 mmol L⁻¹) and Sodium Bicarbonate (1.0 mmol L⁻¹ in 5% acetone) was used as anion carrier and a solution of Tartaric Acid (4 mmol L⁻¹) and Dipicolinic Acid (0.75mmol L⁻¹) was used as cation carrier. A HI 9828 multiparameter probe (Hanna) was used to monitor reactor DO, ORP, temperature and pH.

A technique similar to the one applied by Brotto *et al.* (2015) was used for the emitted N₂O sampling. Emitted N₂O was collected using a modified lab-scale upturned funnel partially submerged in the reactor and a syringe (Figure 1A). The N₂O flux (F) was calculated using Equation 1:

$$F = \frac{Q_{\text{upturned funnel}} \times \Delta[N_2O]}{A_{\text{upturned funnel}}} \quad (1)$$

Where $Q_{\text{upturned funnel}}$ stands for the emerging air flow rate passing through the upturned funnel, $\Delta[N_2O]$ is the difference between the N₂O concentration determined in the reactor and the concentration present in the atmosphere and $A_{\text{upturned funnel}}$ is the superficial area of the upturned funnel.

Dissolved N₂O was collected from the mixed liquor using a syringe and the concentration in the liquid was determined by the headspace gas method (de Mello *et al.*, 2013). Dissolved N₂O concentrations (C) were calculated with Equation 2:

$$C = K_0 \times C_{\text{hs}} + \left[\left(\frac{P}{R \times T} \right) \times (C_{\text{hs}} - C_{\text{ar}}) \right] \quad (2)$$

Where K_0 is the N₂O solubility coefficient (Weiss and Price, 1980), C_{hs} is the N₂O concentration stripped from the liquid (final), P is the atmospheric pressure, R is the universal gas constant, T is the liquid temperature (K) and C_{ar} is the N₂O concentration present in the atmosphere (initial).

Both emitted and dissolved N₂O were stored in glass vials containing a saturated saline solution following Bastviken *et al.* (2010). The N₂O in the vial was recovered and analyzed on

a GC-2014 gas chromatograph with flame ionization (FID) and electron capture detector (GC-ECD) (Shimadzu), using a Porapak-Q packed column, ultrapure N_2 (99,999%) as carrier gas and argon containing 5% CH_4 as make-up.

3. RESULTS AND DISCUSSION

The SBR reached average COD, DOC and TN removal efficiencies of 89, 91 and 79%, respectively, similar to those reported by other authors (Jia, *et al.*, 2012; Rodriguez-Caballero *et al.*, 2015). Jia *et al.* (2012) reported removal efficiencies of 91 and 85% for COD and TN, respectively, in an anaerobic/aerobic SBR designed for simultaneous nitrification and denitrification (SND), with a 6-hour cycle. Rodriguez-Caballero *et al.* (2015) also reported removal efficiencies near 90%, for both COD and TN in a full-scale SBR operated with alternate aerobic and anoxic phases in 4.5-hour cycles.

Despite the high TN removal obtained during this study, a high concentration of NO_2^- at the end of both the aerobic and anoxic phases was noted, with higher values in the aerobic phases (Figure 2). Too low NO_3^- concentrations ($<0.16 \text{ mg N L}^{-1}$) during the cycle were also observed. These results can be an indicator that both nitrification and denitrification were only partial, as reported by other authors (Guo *et al.*, 2009; Stenström *et al.*, 2014; Du *et al.*, 2016). Stenström *et al.* (2014) also observed NO_2^- build-up in a SBR applying both nitrification and denitrification, indicating higher NO_2^- production during nitrification and consumption reduction during denitrification throughout the study. Guo *et al.* (2009) observed NO_2^- concentrations over 25 mg N L^{-1} and NO_3^- concentrations under 6 mg N L^{-1} in an SBR aerobic designed for partial nitrification. In the present study, the NO_2^- accumulation rate reached 96%. Du *et al.* (2016), for an anoxic SBR designed for partial denitrification, reported that NO_2^- accumulated due to decreased NO_2^- enzyme reduction activity. It is known that high nitrification rates may result in decrease of pH, provoking a reaction shift of nitrite and nitrous acid upon pH below 5, which may impact the denitrification (Todt and Dörsch, 2016). However, the pH values measured in the present study were above 5, which may not have been sufficient to increase nitrous acid production. This reinforces the theory that partial nitrification was responsible for the accumulation of nitrite.

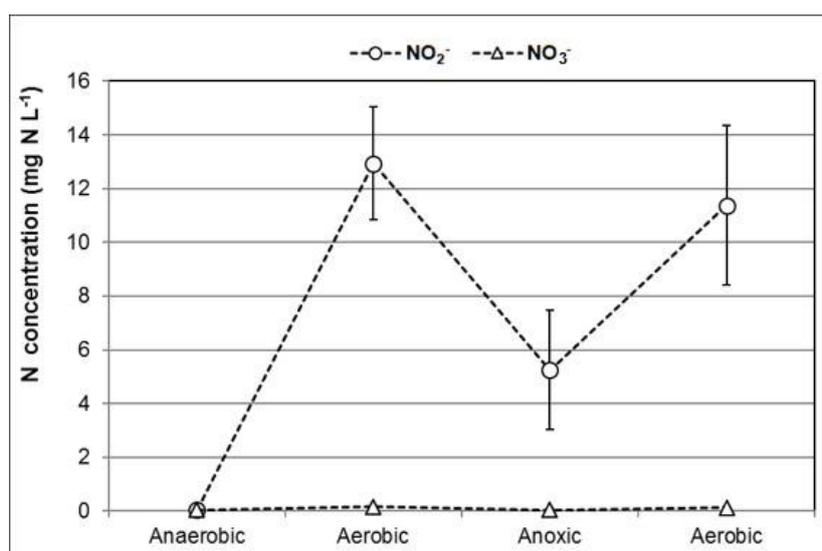


Figure 2. Mean NO_2^- and NO_3^- concentrations (mg N L^{-1}) and their respective standard deviations at the end of each metabolic phase.

Figure 3A presents NO_2^- production (first aerobic phase), consumption (anoxic phase) and build-up (operational cycle) throughout the study. During the first aerobic phase, NO_2^-

consumption rates were high and varied from 43.1 to 63.8 mg N h⁻¹. In the next phase (anoxic), the consumption rates decreased throughout the sampling period, from 40.4 to 20.3 mg N h⁻¹. The combination of high partial nitrification rates (NO₂⁻ generation) during the first aerobic phase with decreased NO₂⁻ consumption in the subsequent anoxic phase led to NO₂⁻ build-up at the end of each operational cycle. The NO₂⁻ build-up rate increased from 29 to 57% at the end of each operational cycle and expresses the NO₂⁻ percentage that was not consumed during the anoxic phase in relation to that produced in the previous phase (aerobic). Wu *et al.* (2011) observed NO₂⁻ accumulation rates near 90% in an anaerobic/aerobic SBR designed for partial nitrification with lower biomass concentrations, while Stenström *et al.* (2014) reported that NO₂⁻ accumulation can lead to increased N₂O production and emission, as observed herein.

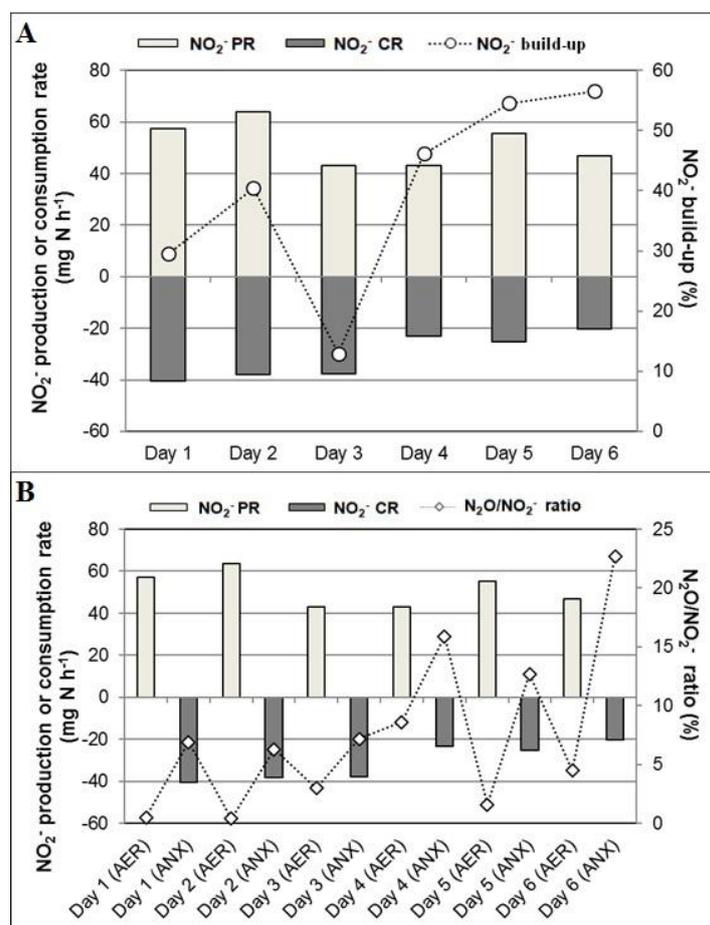


Figure 3. (A) NO₂⁻ production (mg N h⁻¹) during the first aerobic phase, consumption (mg N h⁻¹) in the anoxic phase and build-up (%) during the cycle. (B) NO₂⁻ production (mg N h⁻¹) during the first aerobic phase, consumption (mg N h⁻¹) in the anoxic phase and the N₂O/NO₂⁻ ratio (%) of both phases.

Figure 3B indicates NO₂⁻ production (first aerobic phase) and consumption (anoxic phase) rates and the N₂O/NO₂⁻ ratio for each phase (first aerobic and anoxic) during the study. The increased NO₂⁻ build-up rate coincides with increased N₂O production rate, raising the N₂O/NO₂⁻ ratio (Figure 3B). During the aerobic phase, N₂O production accounted for 0.5 to 8.5% of NO₂⁻ production, where the main production mechanism was nitrification. During the anoxic phase, values were substantially higher and corresponded to the evolution of the denitrification process, ranging from 6.3 to 22.7%. Therefore, denitrification was responsible

for the higher N_2O production in this type of system, being extremely important for the control of the operational conditions of the anoxic phase to mitigate N_2O emissions in the following phases, mainly the aerobic stage. Rodriguez-Caballero *et al.* (2015) reported NO_2^- build-up as a key factor in increasing N_2O production during nitrifying denitrification. Mampaey *et al.* (2016) described the anoxic phase as an important N_2O generation factor, in addition to high NO_2^- concentrations. According to these authors, the anoxic phase was responsible for 70% of N_2O production in a SHARON reactor. Therefore, effective operational parameter control, in order to minimize NO_2^- accumulation and, consequently, N_2O supersaturation in the liquid, is necessary for N_2O emission mitigation measures (Kampschreur *et al.*, 2009; Vasilaki *et al.*, 2019).

The higher N_2O production rate occurring simultaneously with NO_2^- build-up in the system may be associated with a reduction in the reactor biomass (VSS). Figure 4 displays the NO_2^- production (first aerobic phase) and consumption (anoxic phase) rates in parallel with the VSS concentrations in the reactor throughout the study period. A biomass concentration reduction of approximately 30% at the end of the sampling period was observed. The loss of biomass may be related to a mechanical problem in the mixer observed during the fifth week of sampling. The mixer malfunction led to sludge flotation during sedimentation, causing biomass losses through the treated wastewater discharge and, consequently, a sharp reduction in SRT. This event may have caused decreased efficiency of both the nitrification and denitrification processes, resulting in N_2O and NO_2^- accumulation. Wu *et al.* (2011) observed that decreased suspended solid (SS) concentrations in an SBR system favored NO_2^- accumulation. Noda *et al.* (2003) reported higher concentrations of dissolved and emitted N_2O in anoxic and oxide reactors with lower SRT. Other authors have also associated lower SRT with NO_2^- build-up and increased N_2O production and emissions (Hanaki *et al.*, 1992; Kampschreur *et al.*, 2009; Castellano-Hinojosa *et al.*, 2018).

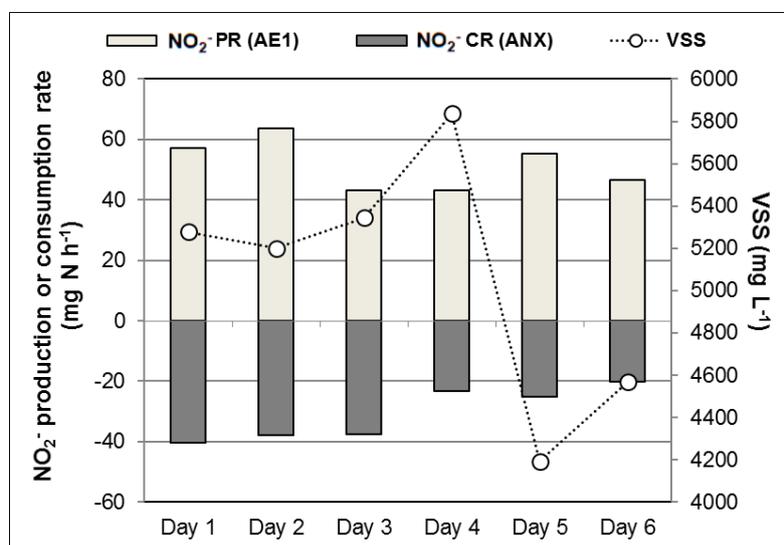


Figure 4. NO_2^- production (mg N h^{-1}) during the first aerobic phase, consumption (mg N h^{-1}) in the anoxic phase and biomass concentrations (expressed as VSS mg N L^{-1}).

As previously reported, solid losses in SBR alongside decreased SRT are likely to favor increasing NO_2^- build-up and N_2O generation. However, an additional effect was observed regarding the magnitude of the N_2O transfer rate from the liquid to the atmosphere during the second aerobic phase. Figure 5 presents the N_2O production rates (from the retained and not-emitted portion) of the first aerobic and anoxic phases in parallel to the maximum N_2O flux at the beginning of the second aerobic phase throughout the study period. The N_2O flux peak

occurred as soon as aeration began during the second aerobic phase, with substantially high values ranging from 706 to 2416 mg N m⁻² h⁻¹. These findings are close to those reported by Ribeiro *et al.* (2017) in a conventional activated sludge WWTP with landfill leachate addition, where a maximum N₂O flux of 1890 mg N m⁻² h⁻¹ was observed, which was correlated to decreased DO concentrations and partial nitrification.

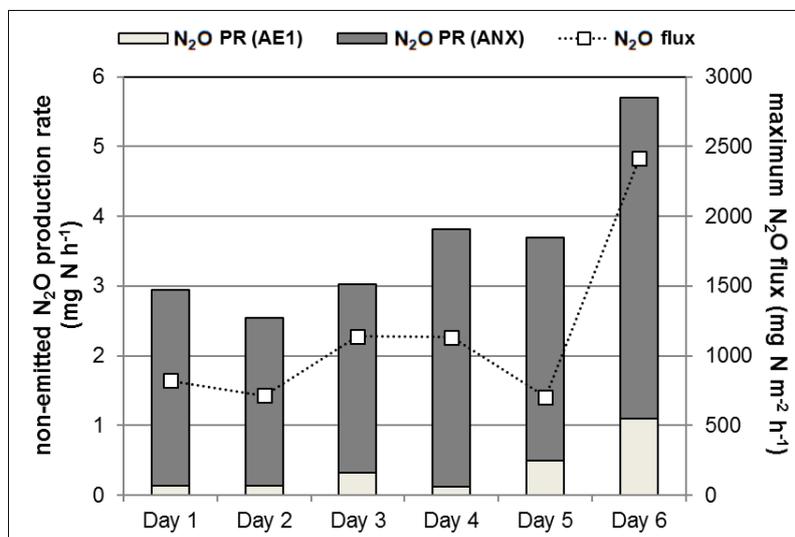


Figure 5. Non-emitted N₂O production rate (mg N h⁻¹) during the first aerobic and anoxic phases and maximum flux emitted during the second aerobic phase (mg N m⁻² h⁻¹).

In the present study, the peaks from the second aerobic phase represented the amount of N₂O produced and retained (not emitted) from the previous phases (first aerobic and anoxic). The same behavior for N₂O/NO₂⁻ accumulation in the liquid phase was observed for the maximum N₂O flux throughout the study period, with an increase in emitted N₂O in parallel with an increased N₂O production rate retained from the previous phases (Figure 5). Other studies have reported the same liquid N₂O accumulation problem during the anoxic phase and its implications for the next aerobic phase (Gustavsson and La Cour Jansen, 2011; Yang *et al.*, 2017; Pijuan *et al.*, 2014; Mampaey *et al.*, 2016). Thus, alternation between the anoxic and aerobic phases can be a negative point for N₂O emission mitigation in wastewater treatment processes applying N removal.

Therefore, operational control in order to favor lower N₂O production during the aerobic phase and its rapid consumption in the subsequent anoxic phase are necessary to mitigate emissions in the following phases, especially in the aerated units. Otherwise, in systems with a subsequent aerobic phase, N₂O produced and not consumed may be emitted. In systems without this subsequent step, N₂O may be emitted during treated wastewater disposal into receiving water bodies. Other operational adjustments, such as NO₂⁻ and SRT control, are extremely important to create favorable conditions for nitrification and denitrification processes without liquid N₂O accumulation and subsequent emission.

4. CONCLUSION

This study correlates N₂O production and emissions with the operational condition of an SBR undergoing anaerobic/aerobic/anoxic/aerobic phases. The main conclusions are:

- Even with controlled DO and ORP, partial nitrification was observed during the first

aerobic phase, resulting in high NO_2^- production. The NO_2^- was not totally consumed during the anoxic phase, also indicating the partial denitrification.

- NO_2^- build-up favored N_2O production during both the aerobic and anoxic phases of the process. The increases observed in both parameters can be associated with decreased biomass concentrations.
- The anoxic phase was responsible for the highest N_2O production rates. The N_2O accumulated during this phase was released during the second aerobic phase, causing emission peaks as soon as aeration began.
- An environment able to sustain complete nitrification and denitrification is required, minimizing NO_2^- accumulation and allowing for rapid N_2O consumption, thus minimizing emissions.

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