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

ARTICLES doi:10.4136/ambi-agua.2634

Received: 16 Jun. 2020; Accepted: 20 Jan. 2021

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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 anaeróbio/aeróbio/anóxico/aeróbio 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 nitrato (NO$_2^-$) e sua influência na produção e emissão de N$_2$O 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 controlado, a primeira fase aeróbia promoveu apenas a nitrificação parcial resultando em acúmulo de NO$_2^-$ (variando de 29 a 57%) e geração de N$_2$O. Este NO$_2^-$ não foi totalmente consumido na fase anóxica subsequente promovendo uma produção ainda maior de N$_2$O pela desnitrificação parcial. Foi observada uma relação direta entre o acúmulo de NO$_2^-$ nessas fases e a produção de N$_2$O. Enquanto na primeira fase aeróbia a razão N$_2$O/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$_2$O durante a fase anóxica do que na primeira fase aeróbia. Com isso, os maiores fluxos de N$_2$O ocorreram na segunda fase aeróbia, variando de 706 a 2416 mg N m$^{-2}$ 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, consequentemente, a emissão de N$_2$O.

**Palavras-chave**: acúmulo de nitrato, 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$_2$O 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$_2$O 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$_2$O is the most emitted gas from anthropogenic sources displaying ozone (O$_3$) depletion potential (Ravishankara et al., 2009). The highest N$_2$O 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$_2$OH), 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$_2$O production is commonly attributed to three pathways: (1) partial nitrification, as a by-product of NH$_2$OH oxidation; (2) nitrifier denitrification, which can occur under oxygen-limiting conditions, as an intermediate product; and (3) heterotrophic denitrification, where N$_2$O is an intermediate product but can be released when the process is incomplete (Duan et al., 2017; Terada et al., 2017). Variations in N$_2$O production and emissions occur according to the type of applied treatment process and configuration and operational parameters (Law et al., 2012).

N$_2$O 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., 2017). Variations in N$_2$O production and emissions occur according to the type of applied treatment process and configuration and operational parameters (Law et al., 2012).
Pijuan et al. (2014) reported N$_2$O 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$_2$O 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$_2$O generation.

Knowledge of parameters that affect N$_2$O 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$_2$O 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](image-url)
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 N$_2$O 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 NO$_2^-$ and NO$_3^-$ analysis. Dissolved and emitted N$_2$O 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). NO$_2^-$ and NO$_3^-$ 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 (0.75 mmol 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 N$_2$O sampling. Emitted N$_2$O was collected using a modified lab-scale upturned funnel partially submerged in the reactor and a syringe (Figure 1A). The N$_2$O flux (F) was calculated using Equation 1:

\[ F = \frac{Q_{\text{upturned funnel}} \times \Delta [\text{N}_2\text{O}]}{A_{\text{upturned funnel}}} \]  

(1)

Where $Q_{\text{upturned funnel}}$ stands for the emerging air flow rate passing through the upturned funnel, $\Delta [\text{N}_2\text{O}]$ is the difference between the N$_2$O 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 N$_2$O 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 N$_2$O concentrations (C) were calculated with Equation 2:

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

(2)

Where $K_0$ is the N$_2$O solubility coefficient (Weiss and Price, 1980), $C_{hs}$ is the N$_2$O 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 N$_2$O concentration present in the atmosphere (initial).

Both emitted and dissolved N$_2$O were stored in glass vials containing a saturated saline solution following Bastviken et al. (2010). The N$_2$O 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 N₂ (99,999%) as carrier gas and argon containing 5% CH₄ 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 NO₂⁻ at the end of both the aerobic and anoxic phases was noted, with higher values in the aerobic phases (Figure 2). Too low NO₃⁻ concentrations (<0.16 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 NO₂⁻ build-up in a SBR applying both nitrification and denitrification, indicating higher NO₂⁻ production during nitrification and consumption reduction during denitrification throughout the study. Guo et al. (2009) observed NO₂⁻ concentrations over 25 mg N L⁻¹ and NO₃⁻ concentrations under 6 mg N L⁻¹ in an SBR aerobic designed for partial nitrification. In the present study, the NO₂⁻ accumulation rate reached 96%. Du et al. (2016), for an anoxic SBR designed for partial denitrification, reported that NO₂⁻ accumulated due to decreased NO₂⁻ enzyme reduction activity. It is known that high nitritation 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](image-url)  
**Figure 2.** Mean NO₂⁻ and NO₃⁻ concentrations (mg N L⁻¹) and their respective standard deviations at the end of each metabolic phase.

Figure 3A presents NO₂⁻ production (first aerobic phase), consumption (anoxic phase) and build-up (operational cycle) throughout the study. During the first aerobic phase, NO₂⁻
consumption rates were high and varied from 43.1 to 63.8 mg N h\textsuperscript{-1}. In the next phase (anoxic), the consumption rates decreased throughout the sampling period, from 40.4 to 20.3 mg N h\textsuperscript{-1}. The combination of high partial nitrification rates (NO\textsubscript{2} generation) during the first aerobic phase with decreased NO\textsubscript{2} consumption in the subsequent anoxic phase led to NO\textsubscript{2} build-up at the end of each operational cycle. The NO\textsubscript{2} build-up rate increased from 29 to 57% at the end of each operational cycle and expresses the NO\textsubscript{2} percentage that was not consumed during the anoxic phase in relation to that produced in the previous phase (aerobic). Wu \textit{et al.} (2011) observed NO\textsubscript{2} accumulation rates near 90% in an anaerobic/aerobic SBR designed for partial nitrification with lower biomass concentrations, while Stenström \textit{et al.} (2014) reported that NO\textsubscript{2} accumulation can lead to increased N\textsubscript{2}O production and emission, as observed herein.

Figure 3. (A) NO\textsubscript{2} production (mg N h\textsuperscript{-1}) during the first aerobic phase, consumption (mg N h\textsuperscript{-1}) in the anoxic phase and build-up (%) during the cycle. (B) NO\textsubscript{2} production (mg N h\textsuperscript{-1}) during the first aerobic phase, consumption (mg N h\textsuperscript{-1}) in the anoxic phase and the N\textsubscript{2}O/NO\textsubscript{2} ratio (%) of both phases.

Figure 3B indicates NO\textsubscript{2} production (first aerobic phase) and consumption (anoxic phase) rates and the N\textsubscript{2}O/NO\textsubscript{2} ratio for each phase (first aerobic and anoxic) during the study. The increased NO\textsubscript{2} build-up rate coincides with increased N\textsubscript{2}O production rate, raising the N\textsubscript{2}O/NO\textsubscript{2} ratio (Figure 3B). During the aerobic phase, N\textsubscript{2}O production accounted for 0.5 to 8.5% of NO\textsubscript{2} 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 N₂O production in this type of system, being extremely important for the control of the operational conditions of the anoxic phase to mitigate N₂O emissions in the following phases, mainly the aerobic stage. Rodríguez-Caballero et al. (2015) reported NO₂⁻ build-up as a key factor in increasing N₂O production during nitrifying denitrification. Mampaey et al. (2016) described the anoxic phase as an important N₂O generation factor, in addition to high NO₂⁻ concentrations. According to these authors, the anoxic phase was responsible for 70% of N₂O production in a SHARON reactor. Therefore, effective operational parameter control, in order to minimize NO₂⁻ accumulation and, consequently, N₂O supersaturation in the liquid, is necessary for N₂O emission mitigation measures (Kampschreur et al., 2009; Vasilaki et al., 2019).

The higher N₂O production rate occurring simultaneously with NO₂⁻ build-up in the system may be associated with a reduction in the reactor biomass (VSS). Figure 4 displays the NO₂⁻ 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 N₂O and NO₂⁻ accumulation. Wu et al. (2011) observed that decreased suspended solid (SS) concentrations in an SBR system favored NO₂⁻ accumulation. Noda et al. (2003) reported higher concentrations of dissolved and emitted N₂O in anoxic and oxide reactors with lower SRT. Other authors have also associated lower SRT with NO₂⁻ build-up and increased N₂O production and emissions (Hanaki et al., 1992; Kampschreur et al., 2009; Castellano-Hinojosa et al., 2018).

![Figure 4](image_url)

**Figure 4.** NO₂⁻ 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 NO₂⁻ build-up and N₂O generation. However, an additional effect was observed regarding the magnitude of the N₂O transfer rate from the liquid to the atmosphere during the second aerobic phase. Figure 5 presents the N₂O production rates (from the retained and not-emitted portion) of the first aerobic and anoxic phases in parallel to the maximum N₂O flux at the beginning of the second aerobic phase throughout the study period. The N₂O 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 N\(_2\)O flux of 1890 mg N m\(^{-2}\) h\(^{-1}\) was observed, which was correlated to decreased DO concentrations and partial nitrification.

In the present study, the peaks from the second aerobic phase represented the amount of N\(_2\)O produced and retained (not emitted) from the previous phases (first aerobic and anoxic). The same behavior for N\(_2\)O/NO\(_2\) accumulation in the liquid phase was observed for the maximum N\(_2\)O flux throughout the study period, with an increase in emitted N\(_2\)O in parallel with an increased N\(_2\)O production rate retained from the previous phases (Figure 5). Other studies have reported the same liquid N\(_2\)O 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 N\(_2\)O emission mitigation in wastewater treatment processes applying N removal.

Therefore, operational control in order to favor lower N\(_2\)O 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, N\(_2\)O produced and not consumed may be emitted. In systems without this subsequent step, N\(_2\)O may be emitted during treated wastewater disposal into receiving water bodies. Other operational adjustments, such as NO\(_2\) and SRT control, are extremely important to create favorable conditions for nitrification and denitrification processes without liquid N\(_2\)O accumulation and subsequent emission.

4. CONCLUSION

This study correlates N\(_2\)O 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
Nitrite build-up effect on nitrous oxide production. The NO$_2^-$ was not totally consumed during the anoxic phase, also indicating the partial denitrification.

- NO$_2^-$ build-up favored N$_2$O 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$_2$O production rates. The N$_2$O 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$_2$O consumption, thus minimizing emissions.

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