Nitrous oxide emission from a moving bed membrane biofilm reactor: the effect of the sludge retention time

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Abstract
The aim of the present study was to investigate the nitrous oxide (N₂O) emissions from a University of Cape Town (UCT) moving bed membrane bioreactor pilot plant. An experimental campaign was carried out during 60 days with three different sludge retention time (SRT). The pilot plant reactor was provided of funnel shape covers that guaranteed gas accumulation in the headspace. The results highlighted that N₂O concentrations significantly increased when the biofilm concentrations increased within the aerobic and anoxic compartments. Furthermore, results have shown an increase of N₂O with the decrease of SRT. Moreover, the MBR tank resulted the key emission source (up to 70% of the total N₂O emission during SRT=∞ period) whereas the highest N₂O production occurred in the anoxic reactor. Moreover, N₂O concentrations measured in the permeate flow were not negligible, thus highlighting its potential detrimental contribution for the receiving water body.

Keywords
Nitrous Oxide; IFAS; UCT; MB-MBR; Activated Sludge

INTRODUCTION
Nitrous Oxide (N₂O) represents a significant greenhouse gas (GHG) with a global warming potential (GWP) 298 times higher than carbon dioxide (CO₂). Due to the reason that many bacteria involved in biological nitrogen removal from the wastewater are capable to produce N₂O (Stenström et al., 2014), in recent years nitrous oxide emissions from wastewater treatment plants (WWTPs) has received increasing attention (Ni and Yuan, 2015; Mannina et al., 2015). Nitrous oxide production is of utmost importance during biological nutrient removal processes (Kampschreur et al., 2009). Indeed, N₂O can be produced by ammonia oxidizing bacteria (AOB) during the nitrification process. Furthermore, N₂O represents also an intermediate product of the heterotrophic denitrification process. Thus, both autotrophic and heterotrophic bacteria can be responsible for N₂O production during BNR (Kampschreur et al., 2009; Chandran et al., 2011; Law et al., 2012). During phosphorus removal process, N₂O production can also occurs (Kampschreur et al., 2009; Zhou et al., 2012).

In the last years many efforts have been spent towards understanding the key mechanisms involved in N₂O production and emission (Kampschreur et al., 2009). As a consequence, several parameters that might favour N₂O production/emission have been identified: low dissolved oxygen concentrations, nitrite accumulation, dynamic conditions as well as low carbon-to-nitrogen (C/N) ratio values during denitrification. Moreover, the technical literature highlights that in processes aimed at the biological nitrogen and phosphorous removal (BNPR), the role of polyphosphate accumulating organisms (PAOs) in the production of N₂O cannot be disregarded (among others, Zhou et al., 2012). Furthermore, some studies have reported that the N₂O emission rates are significantly influenced by the operational conditions of wastewater such as the carbon sources for denitrification (Chiu and Chung, 2000; Zeng et al., 2003; Li et al., 2008; Tallec et al., 2008), and nitrate concentration in the mixed liquid (Park et al., 2000). Ahn et al. (2010) surveyed 12 full scale WWTP across United States and found that N₂O emission factors resulted from 0.01% to 1.8% of influent total nitrogen (TN); furthermore it was observed that the aerobic zones contributed more
N₂O emission than anoxic zones. Among other operational parameters, Sludge Retention Time (SRT) is considered to be capable to influence N₂O production. Indeed, studies carried out on full scale WWTP highlighted the N₂O production increases when the SRT of the WWTP decreases (Kampschreur et al., 2009).

In the last years, the hybrid systems, integrating biofilm within a suspended-growth system, have been proposed for BNPR. Hybrid systems maximize the nitrification by means of high solid retention time (SRT) of the biofilm, but having the potential of operating the suspended growth phase with a relatively short SRT. Moreover, in a hybrid system, biofilm and suspended biomass may have a different role referring to either nitrogen or phosphorus removal. This aspect can be of importance in terms of N₂O emissions from BNPR in hybrid systems. Among the hybrid systems, the joint use of membrane bioreactors (MBRs) and moving bed biofilm reactors (MBBRs) was recently proposed, replacing the secondary settler by means of the membrane module. The latter configuration is usually referred to as moving bed membrane bioreactor (MB-MBR) (Di Trapani et al., 2014). Regarding the biofilm influence in N₂O production, Peng et al (2016), by using a one-dimensional biofilm model, found that the gas production increases when the biofilm thickness increases. Sen et al., (2010) developed a model in order to investigate on the differences in GHG emission between integrated fixed film activated sludge (IFAS) and conventional activated sludge (CAS) and found that the higher buffer on air supply in IFAS that is created when the aeration requirements have to satisfy the need for media mixing to shear the biofilm helps achieve more complete nitrification, thereby lowering the potential for GHG emissions. So far, to the authors knowledge, there are few modeling studies that compare the relative effects of the biofilm and the suspended sludge on N and P removal efficiencies and N₂O emission in a hybrid BNPR system. Therefore, a better understanding of the mechanisms involved in N₂O production from hybrid systems aimed at nutrient removal is still crucial. The key factors affecting N₂O emission in unconventional WWTP (e.g., membrane filtration plants – MBR, moving bed biofilm reactor – MBBR or their combination such as MBBR-MBR) have not yet been identified due to the different behaviour of biomass. In the light of the above discussion, this study aimed to investigate the N₂O production in a hybrid MBBR–MBR system for carbon and nutrients removal. To achieve such goals a University Cape Town (UCT)-MB-MBR was monitored for 75 days and operated at different sludge retention times (SRT).

MATERIALS AND METHODS

Pilot Plant lay-out
Experimentation was carried out on a UCT-MBR pilot plant realized in accordance with draft reported in Figure 1.

Figure 1. Pilot plant layout
Figure 1. Lay-out of the UCT-MBMBR pilot plant (Q_in= Feeding flux; Q_out= Permeate flux; ODR= oxigen depletion reactor; Q_RAS= Recycled activated sludge flux; Q_R1= Anoxic-anaerobic recycle; Q_R2= Aerobic-MBR recycle).
In details, the pilot plant consisted of anaerobic (volume 62 L), anoxic (volume 102 L) and aerobic (volume 211 L) compartments according to the UCT scheme (Ekama et al., 1983; Cosenza et al., 2013). The solid-liquid separation phase was achieved by means of an ultrafiltration hollow fibre membrane module (PURON® Triple bundle Demo Module). The membrane module (nominal pore size 0.03 µm, membrane area 1.4 m²) was located inside a dedicated aerated compartment (referred to as the MBR tank, with a 36 L volume). An oxygen depletion reactor (ODR) allowed oxygen removal in the mixed liquor recycled from the MBR tank to the anoxic tank \((Q_{RAS})\). The membrane was periodically backwashed (every 9 min for a period of 1 min) by pumping a volume of permeate back through the membrane fibres from the Clean In Place (CIP) tank. The influent flow rate was set equal to 20 L h\(^{-1}\) \((Q_{in})\). During pilot plant operation, a 20 L h\(^{-1}\) flow \((Q_{R1})\) was continuously pumped from the anoxic to the aerobic tank. Furthermore, 100 L h\(^{-1}\) \((Q_{R2})\) of mixed liquor were pumped from the aerobic to the MBR tank. A net permeate flow rate of 20 L h\(^{-1}\) was extracted \((Q_{OUT})\) through the membrane. Therefore, the recycled activated sludge \((Q_{RAS})\) from the MBR to the anoxic tank through the ODR tank was equal to 80 L h\(^{-1}\). The anaerobic, anoxic, aerobic and MBR reactors were equipped with specific funnel shape covers that guaranteed gas accumulation in the headspace to capture the produced gas by sampling. Furthermore, the anoxic and aerobic compartments were filled with suspended carriers (Amitech s.r.l.) with a 15 and 40% filling ratio respectively, corresponding to a net surface area of almost 75 m\(^2\) m\(^{-3}\) and 205 m\(^2\) m\(^{-3}\), respectively.

**SRT conditions**

During the first 15 days, the pilot plant was operated in total sludge retention condition, corresponding to an indefinite SRT. From day 16th to day 45th, regular sludge withdrawals were operated from the aerobic reactor in order to set a SRT equal to 30 days. Furthermore, from day 46th to day 60th the sludge withdrawals were boosted as far as the corresponding SRT resulted equal to 15 days.

**Analytical procedures**

During the pilot plant operation, the influent wastewater, the mixed liquor inside the anaerobic, anoxic, aerobic and MBR tanks, and the effluent permeate were sampled and analysed for TSS, volatile suspended solids (VSS), total chemical oxygen demand (COD\(_{TOT}\)), supernatant COD (COD\(_{SUP}\)), ammonium nitrogen (NH\(_4\)-N), nitrite nitrogen (NO\(_2\)-N), nitrate nitrogen (NO\(_3\)-N), total nitrogen (TN), phosphate (PO\(_4\)-P), and total phosphorus (TP), Biochemical Oxygen Demand (BOD\(_5\)). All analyses were performed according to the Standard Methods (APHA, 2005).

**Gas sampling and measurements**

The liquid and gaseous samples were withdrawn from the anaerobic, anoxic, aerobic and MBR tanks and analysed to determine the \(\text{N}_2\text{O}-\text{N}\) concentration. Furthermore, the \(\text{N}_2\text{O}-\text{N}\) fluxes (g\(\text{N}_2\text{O}-\text{N}\) m\(^2\) h\(^{-1}\)) from all the compartments were quantified by measuring the gas flow rates, \(Q_{GAS}\) (L min\(^{-1}\)). \(\text{N}_2\text{O}\) concentration was measured by using a Gas Chromatograph (Thermo Scientific™ TRACE GC) equipped with an Electron Capture Detector (ECD).

**Gas flux assessment**

Gas flow rate, expressed as \(Q_{GAS}\) was measured in accordance to Equation 1.
\[ Q_{\text{GAS}} = v_{\text{GAS}} \cdot A \]  

[1]

where \( A \) represents the outlet section of the sampling funnel (m²) and \( v_{\text{GAS}} \) (m s\(^{-1}\)) is the gas velocity, measured by using an Hot Wire anemometer.

Thus, the gas flux was assessed by applying the Equation 2.

\[ F_{\text{GAS}} = \rho \cdot C \cdot \frac{Q_{\text{GAS}}}{A} \]  

[2]

where \( F_{\text{GAS}} \) represents the gas flux emitted from the sampled reactor (mgN\(_2\)O-N h\(^{-1}\) m\(^{-2}\)), \( \rho \) is the gas density at the recorded temperature (mol m\(^{-3}\)), \( C \) is the measured gas concentration (mg L\(^{-1}\)), \( Q_{\text{GAS}} \) is the gas flow rate (m³ h\(^{-1}\)) and \( A \) represents the emitting surface of each sampled reactor (m²).

**Gas phase sampling**

Gas produced due to biological activities of biomass and accumulated inside the head space of each reactor funnel was collected by withdrawing samples (9 ml) by means of commercial syringes and thus transferred into glass vials where the vacuum was previously created. Samples from anaerobic, anoxic, aerobic and MBR reactors were collected two times per week with tree replicates for each sampling section. Furthermore, samples of gas were analysed with gas chromatograph (GC) equipped with electron capture detector (ECD) to assess the Nitrous Oxide concentration.

**Dissolved phase sampling**

Gas dissolved in the liquid phase measure was conducted on the basis of the head space gas method derived from Kimochi et al. (1998). In detail, 70 mL of supernatant (after 5 min of centrifugation at 8000 rpm) were sealed into 125 mL glass bottles. To prevent any biological reaction, 1 mL of 2N H\(_2\)SO\(_4\) was added. After 24 h of gentle stirring, the bottles were left for 1 h without moving. Thereafter, the gas accumulated in the headspace of the bottles was collected similarly to the gas sampling procedure. Finally, by applying Henry’s Law, the dissolved gas concentration at equilibrium with the headspace gas was calculated. Samples of liquid phase were collected from the same sections discussed for the gas phase with the same sampling frequency; furthermore liquid samples of the MBR permeate were also collected and analysed in order to assess the N\(_2\)O concentration discharged with the pilot plant effluent.

**N\(_2\)O Emission factors**

For each compartment, the evaluation of the N\(_2\)O-N emission factors, expressed as the percentage of N\(_2\)O-N emitted compared to the inlet nitrogen loading rates, was conducted by means of the following Equation 3 derived by Tsuneda et al., 2005:

\[
EF = \frac{N_2O - N_{\text{GAS}}}{HRT_{\text{HS}}} + \frac{N_2O - N_{\text{Dissolved}}}{HRT} \cdot \frac{TN_{\text{IN}}}{HRT} 
\]  

[3]

where EF represents the N\(_2\)O Emission Factor [%]; \( N_2O - N_{\text{GAS}} \) is the nitrous oxide concentration in the gas phase (mg N\(_2\)O-N L\(^{-1}\)), HRT\(_{\text{HS}}\) is the hydraulic retention time of the head space of the sampled reactor, assessed by taking into account the head space volume and the gas flow rate, (h); \( N_2O - N_{\text{Dissolved}} \) is the liquid phase gas concentration (mg N\(_2\)O-N L\(^{-1}\); HRT is the hydraulic retention time of the pilot plant (h) and TN\(_{\text{IN}}\) represents the total nitrogen concentration fed to the pilot plant.
RESULTS AND DISCUSSION
Pilot plant performances

Pilot plant removal performance are briefly summarized in Table 1.

Table 1. Pilot plant removal efficiencies

<table>
<thead>
<tr>
<th>SRT [d]</th>
<th>COD, [%]</th>
<th>COD, [%]</th>
<th>TN [%]</th>
<th>Nitrification [%]</th>
<th>Denitrification [%]</th>
<th>Phosphorus [%]</th>
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<td>59.10</td>
<td>86.24</td>
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Referred to COD concentration in permeate flux; **referred to COD concentration in supernatant of aerobic reactor; ***Total Nitrogen removal efficiency;

Results reported in Table 1 allow to highlight how the SRT variation did not exerted a significant effect on biological performances by passing from indefinite SRT to SRT=30 d. Furthermore, by reducing SRT to 15 d, a slight influence on nitrogen related process was noticed; indeed, nitrification efficiency decreased from 91.29 % to 86.24 % on average passing by SRT=30d to SRT=15 d respectively.

Nitrous oxide

In Figure 2 the nitrous oxide concentration measured in the head space of each reactor and in the liquid concentration are reported.

Figure 2. Nitrous oxide concentration in the head spaces (a) and in the liquid phase (b) during the experimentation
Data reported on figure 2 show the influence exerted by the different SRT in the nitrous oxide production. Indeed, the gas concentration measured in the head space of each reactor increased when the SRT of the pilot plant decreased (Figure 2a). In details, the mean concentrations increased one order of magnitude by passing from SRT indefinite to SRT=30 d. Particularly, the mean concentration measured during SRT=∞ ranged from 2.38 μg N₂O-N L⁻¹ to 6.68 μg N₂O-N L⁻¹ (mean concentration measured in aerobic and anaerobic reactor respectively); while during the experimental period carried out with SRT=30 d the mean concentration ranged from 26.40 μg N₂O-N L⁻¹ to 52.71 μg N₂O-N L⁻¹ (mean concentration measured in aerobic and anaerobic reactor respectively). Furthermore, when the SRT was set equal to 15 d, the mean concentration remained on average similar to values measured in the previous experimental phase except for the MBR reactor. In the membrane tank, a significant reduction of N₂O concentration was observed by passing from SRT=30 d to SRT=15 d, with an average concentration that decreased from 40.63 μg N₂O-N L⁻¹ to 8.93 μg N₂O-N L⁻¹ during SRT=30 d and SRT=15 d respectively.

A similar trend was obtained for the nitrous oxide dissolved in the liquid phase (Figure 2b). Indeed, the decreasing of SRT to 30 d enhanced the N₂O production, with mean concentration increased up to two order of magnitudes. However, by passing to SRT=15 d a significant decrease was noticed in all the reactors except for the anaerobic and anoxic tanks. In details mean concentration measured resulted equal to 67.72 μg N₂O-N L⁻¹, 84.27 μg N₂O-N L⁻¹ and 35.76 μg N₂O-N L⁻¹ for aerobic and MBR reactor and in the permeate flux respectively measured during SRT=30 d; during SRT=15 d mean concentration resulted equal to 31.94 μg N₂O-N L⁻¹, 30.18 μg N₂O-N L⁻¹ and 28.39 μg N₂O-N L⁻¹ for aerobic and MBR reactor and in the permeate flux respectively. On the contrary, mean concentration measured for anaerobic and anoxic reactors increased from 21.87 μg N₂O-N L⁻¹ and 84.71 μg N₂O-N L⁻¹ to 45.61 μg N₂O-N L⁻¹ and 197.97 μg N₂O-N L⁻¹ measured during SRT=30 d and SRT=15 d respectively.

This result is likely due to a twofold reason: i. the activity of suspended biomass that increased due to the decreases of SRT; ii. the contribution of the attached biomass that increased with the increase of its concentration.

Furthermore, it is worth noticing that during the initial 15 days of the SRT=30 d phase no significant variation in N₂O production occurred. Such circumstance is likely due to a time lag necessary for the biological system to cope with the new SRT imposed.

The circumstance that the decrease of SRT resulted in an increase of nitrous oxide production is in agreement with results achieved by other researchers (Kampschreur et al., 2009). Zheng et al. (1994) found that in a continuous nitrifying activated sludge system fed with artificial wastewater, the N₂O production increased when the SRT decreased. Similarly, Noda et al. (2003) found that SRT decreasing resulted in an increase of N₂O production in a continuous activated sludge system fed with real wastewater.

Furthermore, it is important to stress that the N₂O concentration measured in the permeate flux was not negligible. In details, permeate flux concentrations ranged from 0.66 μg N₂O-N L⁻¹ to 187.77 μg N₂O-N L⁻¹ measured during SRT=∞ and SRT=30 d respectively. Thus the N₂O concentration in the permeate flux resulted comparable with concentration measured in the others reactors. Furthermore, as the permeate flux represent the final effluent of the treatment plant, its nitrous oxide content can represents a threat for the receiving water body.

The gas flow rate measurements allowed to assess the flux emitted from each reactor. In figure 3 the flux measured for not aerated (a) and aerated (b) reactors during the experimentation are reported.
Figure 3. Nitrous oxide flux in anaerobic and anoxic reactors (a) and in aerobic and MBR reactors (b) during the experimentation

Data reported in figure 3 allow to observe the difference in emission between aerated and not aerated reactors (up to 2 order of magnitudes). In details, flux emitted from anaerobic and anoxic reactors ranged from $2.03 \, \mu g \, N_2O-N \, m^{-2} \, h^{-1}$ to $7288 \, \mu g \, N_2O-N \, m^{-2} \, h^{-1}$ measured in anaerobic reactor during SRT=$\infty$ and in anoxic reactor during SRT=15 d respectively. While flux emitted from aerobic and MBR reactors ranged from $58.29 \, \mu g \, N_2O-N \, m^{-2} \, h^{-1}$ to $400,240 \, \mu g \, N_2O-N \, m^{-2} \, h^{-1}$ measured respectively in aerobic reactor during SRT=$\infty$ and in MBR reactor during SRT=30 d.

The circumstance that the greater flux emission derives from MBR reactor appears in contradiction with scientific literatures that agree in identify nitrogen transformation process as the key source of $N_2O$ emissions (Kampschreur et al., 2009; Law et al., 2012; Zhao et al., 2014). Indeed, in MBR reactor due to the short (0.36 h) hydraulic retention time (HRT) no biological process should occur, and thus the flux emitted from the MBR reactor should be due only to the $N_2O$ piped ($Q_{R2}$) from the aerobic reactor. However, as the measured flux (as well as liquid and gaseous concentrations) resulted frequently higher in the MBR than in aerobic reactor, it is possible that despite the low HRT nitrous oxide production in MBR reactor occurs.

Furthermore, flux measurements together with concentration measurements allowed to assess the emission factor of each reactor. Results are shown in figure 4.
Data reported in Figure 4a show a quite constant emission during the different periods except for the last days of SRT=30 d period. In details an irregular air supply occurred in that period, with the focus to assess the effect of air supply on both membrane fouling and N₂O stripping, in the aerobic and MBR reactors. Thus the N₂O emission factor assessed during days 41th and 43th resulted equal to 38% and 35% of the influent nitrogen. On average, the mean percentages of N₂O emission resulted equal to 0.13%, 0.21% and 0.76% of influent nitrogen for SRT=∞, SRT=30 d and SRT=15 d respectively (data from days 41th and 43th were excluded for the SRT=30 d period mean value calculation). Thus the decrease of SRT resulted in an increase of emission factor from the pilot plant.

The achieved results are in agreement with other studies that quantified the N₂O emission from WWTPs. Foley and Lantl (2008) derived from 11 full-scale and lab-scale wastewater systems with biological nutrient removal (BNR) a median emission factor of 0.01 kgN₂O–N kg⁻¹ N influent. Foley et al (2010) investigated on the N₂O generation factor across 7 BNR-WWTP and found an high variability (in the range 0.006–0.253 kgN₂O–N kg⁻¹ N denitrified (average: 0.035 ± 0.027).

Furthermore, data reported in figure 4 b, c, and d allow to observe the contribution of each reactor to the emission during each experimental period. In details, the decrease of SRT resulted in an increase of aerobic reactor contribution in detriment of MBR reactor. Indeed, aerobic contribution increased from 11.72% to 39.75% by reducing SRT and simultaneously MBR reactor contribution decreased from 70.42% to 46.73%. Such result is likely due to a limitation exerted by the SRT decreasing to the whole nitrogen removal process. Indeed, as noticeable from data reported in table 1, SRT decrease resulted in a slight decrease of TN removal efficiency, moreover the lowest efficiency of nitrification and denitrification were measured in correspondence of SRT=15 d period.
The strong influence exerted by the nitrogen forms in the nitrous oxide production was also investigated in the present study. In figure 5, the influence exerted by nitrite concentration in the N\textsubscript{2}O concentration in the head space of anoxic reactor is reported.

Data reported in figure 5 allow to observe that limitation to nitrification and denitrification occurred. Indeed the presence of nitrite in the aerobic reactor (figure 5a) prove that a partial limitation of nitrification occurred. Furthermore, the finding that N\textsubscript{2}O concentration increase with nitrite concentration, and thus with nitrification limitation, is in agreement with literature that identify limitation in the nitrification process as one of the key factor involved in nitrous oxide production (Kampschreur et al., 2009).

The presence of nitrite also in the anoxic reactor (Figure 5b) up to 3 mg NO\textsubscript{2}-N L\textsuperscript{-1} demonstrates that also the denitrification process resulted limited likely by the decrease of SRT. Furthermore, the high accordance (R\textsuperscript{2}=0.84) existing between N\textsubscript{2}O-N concentration in the head space and nitrite dissolved concentration in anoxic reactor suggest that the incomplete denitrification resulted as the main source of N\textsubscript{2}O production. Such result is consistent with data reported in figure 4 b, c and d; indeed the anoxic reactor contribution, where denitrification occurs, to the total nitrous oxide emission increased during the experimentation from 2.46% to 4.97% to 10.07% during SRT=\infty, SRT=30 d and SRT=15 d respectively. It has to be stressed that the algorithm applied for the emission factor assessment derived by Tsuneda et al. (2005) is strongly influenced by the measured air flux. The circumstance that the anoxic contribution increased despite no air supply is present in the anoxic reactor prove a significant increase in N\textsubscript{2}O production in the anoxic reactor.

**CONCLUSIONS**

The current study explored the influence of SRT in a UCT-MB-MBR pilot plant on the N\textsubscript{2}O formation. The pilot plant was fed with real wastewater collected from the urban sewage. Furthermore the growth of attached biofilm was enhanced by filling anoxic and aerobic reactors with suspended carriers (Amitech s.r.l.) with a 15 and 40% filling ratio respectively. The SRT decrease resulted in an increase of nitrous oxide production and emission. The highest N\textsubscript{2}O concentration was found in the anoxic off-gas. While the highest emitted N\textsubscript{2}O flux was related to the MBR tank (up to 400 mg N\textsubscript{2}O-N m\textsuperscript{-2} h\textsuperscript{-1}). This result is likely due to the higher aeration provided in the MBR to mitigate the membrane fouling as well as to the occurrence of N\textsubscript{2}O formation process in the MBR reactor.

The emission factor assessment proved an increase of anoxic and aerobic contribution to the total emission. Such result was attributed to limitation to nitrification and denitrification process that occurred with the decrease of SRT. The nitrite presence in anoxic and aerobic reactor was found in good agreement with N\textsubscript{2}O concentration measured. Moreover, the N\textsubscript{2}O concentration measured in the permeate flow resulted not negligible (up to 187 \mu g N\textsubscript{2}O-N L\textsuperscript{-1}).
The SRT was thus confirmed to be a key parameter in order to control the nitrous oxide formation and emission from the wastewater treatment plant.

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