RESEARCHING NEW WAYS TO REDUCE N\(_2\)O EMISSION FROM A GRANULAR SLUDGE SEQUENCING BATCH REACTOR TREATING DOMESTIC WASTEWATER UNDER SUBTROPICAL CLIMATE CONDITIONS

Gilberto C. Daudt\(^*\), Jéssica A. Xavier\(^1\), Bianca Meotti\(^1\), Lorena B. Guimarães\(^1\) and Rejane H. R. da Costa\(^1\)

\(^1\) Universidade Federal de Santa Catarina, Centro Tecnológico, Departamento de Engenharia Sanitária e Ambiental, Florianópolis, SC, Brasil. E-mail: gilberto.cd@ufsc.br, ORCID: 0000-0003-3167-1985; jes_antunes@hotmail.com, ORCID: 0000-0002-9637-9734; meottibianca@gmail.com, ORCID: 0000-0002-8449-0910; lobguimaraes@gmail.com, ORCID: 0000-0001-9831-3547; rejane.costa@ufsc.br, ORCID: 0000-0002-2429-5777

(Submitted: November 8, 2017; Revised: May 24, 2018; Accepted: July 10, 2018)

Abstract - N\(_2\)O emissions from wastewater treatment plants have become an important issue, since this compound is a significant greenhouse gas that affects the sustainability of sewage treatment. This work aimed to investigate and to reduce N\(_2\)O emission from a pilot-scale aerobic granular sludge sequencing batch reactor (AGS-SBR) operated for carbon and nitrogen removal from domestic wastewater under subtropical climate condition. Three operational strategies (S-I, S-II and S-III) with different anoxic phase durations were compared regarding treatment efficiency and N\(_2\)O emission. For all the studied strategies, volatile suspended solids (VSS) was between 1.0 and 1.2 g/L. S-III, with the longest anoxic phase, obtained the highest biological oxygen demand (BOD) and NH\(_4\)\(^+\)–N removal efficiencies (86% and 84%, respectively), the lowest N\(_2\)O emission factor (16.99 gN\(_2\)O-N/person·year) and the lowest total nitrogen (TN) to N\(_2\)O conversion ratio (0.47%). The results indicated that the extension of the anoxic phase was an effective way to significantly reduce N\(_2\)O emission and to improve treatment efficiency.

Keywords: Nitrous oxide; Aerobic granular sludge; Domestic wastewater; Sequencing batch reactor; Subtropical climate.

INTRODUCTION

Production and emission of greenhouse gases (GHG) from wastewater treatment plants (WWTP) is a very important issue, which is becoming increasingly significant (Foley et al., 2010; Mannina and Cosenza, 2015). In particular, N\(_2\)O emission in wastewater treatment systems with biological nutrient removal deserves special attention, since this compound is one of the main GHG, and its global warming potential is about 300 times higher than that of CO\(_2\) (Yang et al., 2013).

For over a century, the conventional activated sludge process has been a standard model of biological wastewater treatment systems. However, this technique has as main drawbacks the poor settling properties of the biomass, which is in the form of flocs and can compromise the quality of the final effluent, and the requirement of large areas to place the secondary clarifiers (Liu and Tay, 2004). Sequencing batch reactors (SBR) with aerobic granular sludge (AGS) are presented as a promising option for the biological treatment of domestic and industrial effluents, due to...
the efficiency and robustness of this type of system (Di Bella and Torregrossa, 2013; Moreira et al., 2015; Pronk et al., 2015). With this technique, it is possible to obtain high removal rates of organic matter and nutrients in a single reactor, producing a final effluent with high quality (De Kreuk et al., 2005; Liu et al., 2015). However, the dynamics of nitrogen removal in this process, including nitrous oxide emission, are not completely understood; thus, further research is required.

In the current scientific literature, there are few studies reporting N\textsubscript{2}O emission by biological treatment systems using aerobic granular sludge. The few studies that address this topic are generally related to systems fed with synthetic wastewater (Rathnayake et al., 2013; Wei et al., 2014), being even more scarce reports concerning the emission of N\textsubscript{2}O by granular sludge fed with real wastewater (Castro-Barros et al., 2015), especially under tropical and subtropical climate conditions.

Wide variations concerning N\textsubscript{2}O emissions have been reported in scientific literature, as it can be affected by many factors. Conversions of influent nitrogen to N\textsubscript{2}O reported by Kampschreur et al. (2009) ranged from 0.001 to 14.6%. Moreover, a national survey conducted by Ahn et al. (2010) in the United States listed conversions ranging from 0.01 to 1.8% of the influent nitrogen. The N\textsubscript{2}O emission factor from a municipal activated sludge WWTP reported by Daelman et al. (2013) was 163.2 g N\textsubscript{2}O–N/person·year, which is 80 times higher than the EF proposed by the IPCC (2006). Thus the importance to estimate a N\textsubscript{2}O emission factor for each specific condition.

With this work, the aim was to advance the knowledge concerning the behavior and the rates of N\textsubscript{2}O emission in a pilot-scale SBR with AGS fed with domestic wastewater under subtropical climate conditions. The objectives of this research were: i) to develop aerobic granular sludge in a pilot SBR fed with municipal sanitary wastewater; ii) to evaluate the performance of the treatment; and iii) to monitor, to quantify and to compare N\textsubscript{2}O emissions from the SBR operated under three cycle configurations.

**MATERIALS AND METHODS**

**Pilot reactor operation and cycle configurations**

The pilot reactor studied was an acrylic bubble column 2.18 meters high and with a 25 cm internal diameter, which worked as a SBR. The working volume was 98 L and the reactor outlet pipe provided the exchange of 56% of the liquid volume, in order to treat 55 L of influent during each cycle. The reactor was operated at room temperature (21–26°C) without pH control.

The reactor was operated under three different strategies: Strategy I (S-I), Strategy II (S-II), and Strategy III (S-III), each one with different anoxic phase duration. The cycle phases configuration of the three strategies are shown in Figure 1. During the anoxic phase of S-III, in order to maintain the sludge dispersed through the liquid column when aeration was off, there were aeration pulses of 10 seconds each 15 minutes, which were short enough to keep a very low dissolved oxygen (DO) concentration in the mixed liquor. The variation of the anoxic and aeration

![Figure 1. SBR cycle phases configuration during S-I, S-II and S-III.](image-url)
phase durations was tested to verify its influence on the treatment efficiency and on the N\textsubscript{2}O emission. The SBR cycle configuration corresponded to a hydraulic retention time (HRT) of 7.13 hours for S-I and S-II, and 10.69 hours for S-III.

These 3 cycle configurations were adopted in order to favor granular sludge formation and accumulation in the reactor, and to compare the SBR performance under different regimes. The intention was to obtain aerobic granules with a gradient of oxygen through them, providing aerobic/anoxic layers and the occurrence of simultaneous nitrification-denitrification (SND).

In this study, real domestic wastewater was used to feed the system (influent characterization is in Table 1). The wastewater was collected from an inspection chamber of the public sewage system, located in the city of Florianópolis, state of Santa Catarina, south of Brazil (27°36'12.7"S; 48°31'14.9"W). During the SBR start-up period, wash-out conditions were applied to favor the aerobic granules formation. The selective conditions applied were the shear force caused by the aeration, maintained at 32 L\textsubscript{air}/min, and the reduced settling time (15 minutes). The settling velocity imposed to the biomass to be retained inside the system was 7.47 cm/min.

Analytical procedures
The sludge settleability was assessed through sludge volume index (SVI), which was calculated at times of 5, 10 and 30 minutes (SVI\textsubscript{5}, SVI\textsubscript{10} and SVI\textsubscript{30}, respectively) (Schwarzenbeck et al., 2004). Granulometric analyses were performed through laser diffraction (Mastersizer 2000 – Malvern Instruments, UK), and optical microscopy (Olympus BX40, Japan) was used to observe granules formation. According to Liu et al. (2010), the sludge is considered granular when at least 50% of the biomass presents diameter over 200 μm.

The performance of the wastewater treatment was analyzed in terms of carbon and nitrogen removal. Biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) were quantified according to Standard Methods (APHA, 2005). Nitrite and nitrate were quantified by ion chromatography (DIONEX ICS-500). Total nitrogen (TN) and ammonium (N-NH\textsubscript{4}+) were quantified using Hach® test kits (High Range Nitrogen-Ammonia Reagent Set: #2606945; High Range Total Nitrogen Reagent Set: #2714100). A multiparameter probe (YSI 6820, USA) was used to measure dissolved oxygen (DO) concentration regularly.

\textbf{N\textsubscript{2}O emission quantification}
For purposes of quantifying N\textsubscript{2}O emission, a graph of N\textsubscript{2}O concentration versus time was plotted, and the area under the curve was calculated using a definite integral. The total amount of N\textsubscript{2}O released in one SBR cycle was calculated according to Eq. 1.

\[
m_{\text{N}_2\text{O}} = \int \left( C_{\text{N}_2\text{O}} \times dt \right) \times Q_{\text{air}} \times \frac{1}{60}
\]

where \(m_{\text{N}_2\text{O}}\) is the total amount of N\textsubscript{2}O released in one SBR cycle (g N\textsubscript{2}O); \(\int C_{\text{N}_2\text{O}} \times dt\) is the integral of the variation of N\textsubscript{2}O concentration over time (g N\textsubscript{2}O·s/L\textsubscript{air}); \(Q_{\text{air}}\) is the air flow rate (L\textsubscript{air}/min).

The total amount of nitrogen released to the atmosphere in a SBR cycle was calculated according to Eq. 2.

\[
m_{\text{Nal}} = \left( TN_{\text{i}} - TN_{\text{E}} \right) \times V_e \times \frac{1}{1000}
\]

where \(m_{\text{Nal}}\) is the amount of released nitrogen in one SBR cycle (g N); \(TN_{\text{i}}\) the total nitrogen influent concentration (mg N/L); \(TN_{\text{E}}\) the total nitrogen effluent (mg N/L); \(V_e\) is the volume of wastewater treated in one SBR cycle (L).

The conversion of total influent nitrogen to N\textsubscript{2}O was calculated by relating the amount of N\textsubscript{2}O-N released in a SBR cycle, the TN influent concentration and the volume of wastewater treated in a SBR cycle (Eq. 3).
where Conversion\textsubscript{\textit{N\textsubscript{2}O\textsubscript{inf}→N\textsubscript{2}O-N}} is the fraction of influent nitrogen converted to N\textsubscript{2}O (%); \textit{m\textsubscript{N\textsubscript{2}O-N}} is the amount of N\textsubscript{2}O released in one SBR cycle, expressed in terms of nitrogen (g N\textsubscript{2}O-N).

The N\textsubscript{2}O Emission Factor (EF) was calculated by relating the amount of released N\textsubscript{2}O, the daily per capita wastewater generation and the volume of wastewater treated in one SBR cycle, according to Eq. 4.

\[ \text{EF} = \frac{\textit{m\textsubscript{N\textsubscript{2}O-N}} \times \textit{Q}_{\text{pe}} \times 365}{\textit{V}_{\text{te}}} \]  

where EF is the N\textsubscript{2}O Emission Factor (g N\textsubscript{2}O-N/person·year); \textit{m\textsubscript{N\textsubscript{2}O-N}} is the amount of released N\textsubscript{2}O, expressed in terms of nitrogen (g N\textsubscript{2}O-N); \textit{Q}_{\text{pe}} is the daily per capita wastewater generation (L/person·d).

The N\textsubscript{2}O Flow-Based Emission Factor (FBEF) was calculated by relating the amount of released N\textsubscript{2}O and the volume of wastewater treated in one SBR cycle, according to Eq. 5.

\[ \text{FBEF} = \frac{\textit{m\textsubscript{N\textsubscript{2}O-N}}}{\textit{V}_{\text{te}}} \]  

where FBEF is the N\textsubscript{2}O Flow-Based Emission Factor (g N\textsubscript{2}O-N/L).

**Microbiological procedures**

Next-generation sequencing and fluorescent \textit{in situ} hybridization (FISH) techniques (van Loosdrecht et al., 2016) were used to investigate the microbial community of the granular sludge samples from S-I, S-II and S-III. DNA sequencing was performed using MiSeq\textsuperscript{®} Illumina technology for sequencing by synthesis (SBS). The DNA was extracted from biomass samples using a MoBio PowerBiofilm\textsuperscript{TM} DNA extraction kit (MoBio Laboratories, USA). The rRNA 16S V3/V4 region was amplified using the 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) primers, with Illumina adapters, required for sequencing. The amplification was performed in 35 cycles at 50°C of annealing temperature, where each sample was amplified in triplicate. The sequencing was performed in Illumina MiSeq\textsuperscript{®}, using the V2 kit, with a single-end 300 runs. The system guaranteed the reading of 100,000 sequences with sampling taxonomic identification and quantification of the number of sequences obtained from each taxon. OTU Picking was performed using BLASTN 2.2.28 against GreenGenes 13.8 database. To attribute taxonomy, only sequences with hits higher than 99% of identity in alignment were considered.

The fluorescent \textit{in situ} hybridization (FISH) technique was used to investigate the microbial community of the granular sludge samples from S-I, S-II and S-III. Granular sludges sampled from the reactor were fixed in 4% paraformaldehyde solution for 2–3 h at 4°C. The sludge samples were rinsed twice with phosphate-buffered saline (PBS) and then dehydrated by successive 50%, 80%, and 98% ethanol washes (Amann, 1995). \textit{In situ} hibrizations were performed using the specific probes NSO190, Ntspa662 and PAE997 for ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and \textit{Pseudomonas} genus, respectively. Oligonucleotides were synthetized and fluorescently labeled with a hydrophilic sulfoindocyanine dye (Cy-3) at the 5’ end. Details on oligonucleotide probes are available at probeBase.

**RESULTS AND DISCUSSION**

**Biomass characteristics and composition**

AGS was successfully cultivated in the SBR fed with domestic wastewater containing low concentration of organic substrate, without adding an external carbon source and without biomass inoculation. The average biomass concentrations in the mixed liquor during S-I, S-II and S-III were 1100 mg/L, 1200 mg/L, and 1050 mg/L, respectively. Considering granules to be the particles with diameters between 0.2 and 5.0 mm (Liu et al., 2010), granulometric analyses indicated average granular biomass fractions of 66%, 32% and 59% during S-I, S-II and S-III, respectively.

Initially, before the granules formation, all particles presented diameters below 200 µm. On the 16th day, 83% of the biomass reached diameters higher than 200 µm, and 46% of the particles were greater than 600 µm. The granular biomass varied throughout the studied strategies, showing mean diameters of 427±89, 265±51 and 292±54 µm for S-I, S-II and S-III, respectively.

According to de Kreuk et al. (2007), the biomass is considered to be predominantly granular when at least 50% of the biological aggregates present diameters superior to 200 µm. Therefore, the sludge was considered predominantly granular during S-I and S-III. However, the same authors noted that other characteristics, such as SVI, must also be considered in granular systems evaluations.

During S-I, SVI\textsubscript{5}, SVI\textsubscript{10} and SVI\textsubscript{30} presented the highest variation. This fact can be attributed to biomass instability during the granules formation and stabilization, as granulation is a gradual process comprising three stages: (i) sludge acclimation, (ii)
sludge aggregation and (iii) granules maturation (Wang et al., 2005). Low SVI values and $\text{SVI}_{20}/\text{SVI}_{10}$ ratios close to 1 are associated with a denser and more compact biomass, with good settling properties. The mean $\text{SVI}_{20}$ values were 126, 118 and 70 mL/g for S-I, S-II and S-III, respectively. In another study with AGS in SBR operated under similar conditions, Wagner and Costa (2013) verified that $\text{SVI}_{20}$ decreased gradually and stabilized at 53 mL/g after 100 days of operation. During S-II, $\text{SVI}_{20}$ and $\text{SVI}_{10}$ values were closer than under S-I, indicating an improvement in the settleability. The $\text{SVI}_{10}/\text{SVI}_{10}$ ratio remained 0.8 from the 149th until the 230th day, indicating an improvement in granular structural stability and biomass compactness, even with biomass concentration variations in the reactor.

S-III, which presented the longest anoxic phase, showed the closest values among SVI, and the average $\text{SVI}_{20}/\text{SVI}_{10}$ ratio was 0.88±0.09, reaching 1.0 on the 286th and 356th days. According to de Kreuk et al. (2007), the $\text{SVI}_{20}/\text{SVI}_{10}$ ratio gives excellent information regarding the granular fraction of the biomass. The higher the ratio, the better the granule settleability and compactness. Furthermore, the $\text{SVI}_{20}/\text{SVI}_{10}$ ratio also indicates the granulation process status (Liu and Tay, 2007). These authors consider the granulation process to be completed when the $\text{SVI}_{20}/\text{SVI}_{10}$ ratio reaches 0.9. Therefore, even with the diameter decrease verified in S-II and S-III, the granule settleability and compactness improved. According to Liu and Tay (2007), a higher granule size does not guarantee a better settleability, while the SVI is directly related to sludge density. This means that aerobic granulation should not be restricted only to the granule size increase, but also to the improvement in the biomass compactness and settleability.

Wastewater treatment performances

The effluent concentrations and the removal efficiency in terms of carbon and nitrogen verified for S-I, S-II and S-III are presented in Table 2. Although there were fluctuations in the soluble COD influent, the effluent concentrations did not show considerable variations. Under S-I, the COD$_{\text{soluble}}$ removal efficiency was 79%, with a mean effluent concentration of 52 mg/L. During S-II, the removal efficiency was 70%, with the SBR effluent presenting an average concentration of 50 mg/L. In S-III, the COD$_{\text{soluble}}$ removal rate was 68% and the effluent concentration was 58 mg/L.

Organic matter removal was also analyzed through BOD concentration. As observed with COD, influent BOD varied since the system was fed with real wastewater. An improvement in the BOD removal was observed over time, from 69 to 86%, and effluent concentrations from 106 to 31 mg/L. Regarding Brazilian national regulations CONAMA 430/2011, which require at least 60% removal or an effluent BOD concentration below 120 mg/L, all strategies met the quality criteria for carbon removal. The BOD removal improvement that was observed in S-III might also be related to the change in the HRT, which went from 7.13h (S-I and S-II) to 10.69h (S-III).

In terms of nitrogen removal, under S-I and S-II the NH$_4^+$–N average removal efficiency was below 60%. The longest aeration phase, applied in S-III, favored ammonium removal, achieving a stable and effective removal of 80% under this operational condition. In fact, as can be seen in Figure 2, which shows the pH profiles during the GSBR cycles, S-III presented the highest decrease in pH during the aerobic phase, indicating the occurrence of a more intense nitrification process in relation to S-I and S-II. As with the BOD removal, the NH$_4^+$–N removal improved with the increase in the HRT from S-I and S-II to S-III. This fact is consistent with the results presented by Wagner and Costa (2013), who observed a significant increase in the NH$_4^+$–N removal when the HRT went from 7.5h to 10h, when operating a SBR under conditions similar to the present study.

During aeration, due to nitrification, there was a progressive increase of nitrite formation, with mean effluent concentrations of 5.9 (S-I), 9.7 (S-II) and 14.5 (S-III) mg NO$_2^-$–N/L. Nitrate was formed in trace concentrations in S-I and S-II, remaining at low levels during these operational strategies, in a range between 0.1 and 0.4 mg NO$_3^-$–N/L. During S-III, a higher nitrate formation was observed, with a mean effluent concentration of 4.15±1.29. These results indicate the occurrence of incomplete nitrification in all conditions.

Table 2. Effluent concentrations and removal efficiencies verified for S-I, S-II and S-III.

| Strategy | Duration | Effluent (mg/L) | Removal efficiency (%) | Removal efficiency (%) |
|----------|----------|----------------|------------------------|------------------------|
| I        | 143 days | COD$_{\text{total}}$ 150±63 | 69±13 | 89±34 | 79±14 | 136±23 | 64±6 |
|          |          | COD$_{\text{soluble}}$ 52±12 | 79±5 | 50±13 | 70±12 | 58±9 | 68±4 |
|          |          | BOD 106±31 | 69±6 | 70±28 | 76±7 | 31±7 | 86±3 |
|          |          | NH$_4^+$–N 24±11 | 52±15 | 20±11 | 57±18 | 8±4 | 84±8 |
|          |          | NO$_2^-$–N 5.9±2.5 | - | 9.7±3.7 | - | 14.5±5.7 | - |
|          |          | NO$_3^-$–N 0.14±0.05 | - | 0.3±0.14 | - | 4.15±1.29 | - |

Brazilian Journal of Chemical Engineering, Vol. 36, No. 01, pp. 209 - 220, January - March, 2019
tested, with higher nitrite accumulation and nitrate formation at the longest aeration period. The extension of the anoxic phase of the cycle did not show as great an influence on nitrite accumulation as the extension of aeration phase.

Nitrite accumulation in reactors with aerobic granules has been reported by some authors (Yang et al., 2013; Isanta et al., 2012; Coma et al., 2012), including conditions of low-strength wastewater (Wang et al., 2007; Figueroa et al., 2008). Although nitrite-oxidizing bacteria (NOB) have their activity decreased by low DO concentration, in the present study the cause of partial nitrification was not low DO in the mixed liquor, since DO remained close to 8 mg/L throughout the aeration phase of the operational cycle, due to the high level of aeration required. Typical pH and DO cycle profiles can be seen in Figure 2.

In this study, the temperature might have been one important factor that contributed to nitrite accumulation, since it reached values up to 27°C inside the reactor. The maximum specific growth rate of ammonium-oxidizing bacteria (AOB) is higher than that of NOB at temperatures above 15°C (Bérnet and Spérandio, 2009), which can favor nitrite accumulation. In fact, this is the basis of SHARON technology, which consists of a chemostat reactor operated at 30°C with a low HRT, to favor AOB growth and NOB washout, achieving partial nitrification (Hellinga et al., 1998). The present research showed that, when the HRT increased from 7.13h (S-I and S-II) to 10.69h (S-III), there was a higher nitrate production, i.e., a more complete nitrification process.

The solids retention time (SRT) is another parameter that can influence nitrite accumulation. The calculated SRT were 14, 15 and 9 days for S-I, S-II and S-III, respectively. However, in the present research, nitrite accumulation might have been more strongly associated with the cycle duration than with the SRT. The cycle duration, which was extended from 4h (S-I and S-II) to 6h (S-III), favored the occurrence of nitrification, as there was a longer time to allow ammonium oxidation.

![Figure 2. Profiles of pH and DO concentration during a typical SBR cycle of each strategy (S-I, S-II and S-III).](image)
Besides temperature and cycle duration, another factor that could contribute to nitrite accumulation is AOB and NOB stratification and the existence of an oxygen gradient in the aerobic granules. The presence and predominance of AOB colonies in the outer layer of the granules could favor nitritation, since they are in a more beneficial position for oxygen consumption than NOB, which are present in the inner layers (Poot et al., 2016; Guimarães et al., 2017). Many studies have tried to achieve partial nitrification, while this study showed feasible SBR operational conditions in tropical and subtropical climate for this.

The nitrite route has several advantages, including a lower oxygen requirement for nitrification (25% less), lower organic carbon consumption in denitrification (40% less) and lower sludge production (Van Loosdrecht and Jetten, 1998). These advantages are even more notable in the case of nitrogen-rich wastewater with a low organic carbon content. However, one of the main concerns related to nitrite accumulation is \( \text{N}_2\text{O} \) production. Several studies have shown that nitrite accumulation is usually accompanied by higher \( \text{N}_2\text{O} \) emissions (Itokawa et al., 2001; Kampschreur et al., 2008).

\( \text{N}_2\text{O} \) emissions

The variation of \( \text{N}_2\text{O} \) emitted in one typical cycle of the SBR in each operational condition tested is shown in Figure 3. For all of the studied strategies, \( \text{N}_2\text{O} \) emission was not constant during the cycle phases. During S-I and S-II, the emission of \( \text{N}_2\text{O} \) started at the beginning of the aerobic phase, and the peak concentration of \( \text{N}_2\text{O} \) occurred in the early moments of aeration, between 2’ and 2’30”, and then decreased until it ceased. During S-III, the \( \text{N}_2\text{O} \) emission pattern was similar to S-I and S-II. However, some \( \text{N}_2\text{O} \) emission was also observed during the aeration pulses applied in the anoxic phase of this strategy. The maximum emissions were 0.90, 0.36 and 0.12 mg \( \text{N}_2\text{O}/\text{s} \) for S-I, S-II and S-III, respectively, showing that, when extending the anoxic phase, a lower \( \text{N}_2\text{O} \) emission peak was observed.

The fact that the peak emission of \( \text{N}_2\text{O} \) occurred at the beginning of the aerobic phase does not mean that this is the moment of greatest \( \text{N}_2\text{O} \) production. In fact, the emission pattern suggests that denitrification was possibly the major source of \( \text{N}_2\text{O} \) generation, probably due to the occurrence of an incomplete denitrification process. Moreover, there seems to be no \( \text{N}_2\text{O} \) formation during the occurrence of nitrification. Yang et al. (2013), who investigated \( \text{N}_2\text{O} \) emission by a single stage reactor with partial nitrification/anammox, suggested that \( \text{N}_2\text{O} \) emitted during aeration is produced by microorganisms during the anoxic phase of the reactor cycle. Since there is no air flow during the anoxic phase, \( \text{N}_2\text{O} \) is retained in the system and accumulates during this step. Mello et al. (2013) investigated the emission of \( \text{N}_2\text{O} \) by an activated sludge treatment plant with intermittent aeration and found that less than 1% of the produced \( \text{N}_2\text{O} \) was released in the absence of aeration.

Therefore, the \( \text{N}_2\text{O} \) that accumulated in the mixed liquor during the anoxic phase is released when aeration starts, causing a peak in the concentration of \( \text{N}_2\text{O} \) emitted at the beginning of the aerobic phase. After a few minutes, all the accumulated \( \text{N}_2\text{O} \) ends up being released to the atmosphere and the emission falls to values near zero.

There are several factors that suppress \( \text{N}_2\text{O} \) production, and consequently the \( \text{N}_2\text{O} \) emission, under the aeration phase of the reactor cycle. Kampschreur et al. (2008) found that high concentration of DO during nitrification prevented \( \text{N}_2\text{O} \) production by microorganisms. In the present study, DO values during the aerobic phase were between 8.0 and 8.8 mg/L, which could prevent the formation of \( \text{N}_2\text{O} \) in this period.

Conversely, there are aspects that contribute to \( \text{N}_2\text{O} \) emission, such as the occurrence of nitrite accumulation during nitrification, which can later be
converted to N₂O during denitrification. Furthermore, if the denitrification process is incomplete, the N₂O formed might not subsequently be converted to N₂, causing a higher net N₂O generation. Besides that, incomplete denitrification can lead to a higher nitrite concentration in the mixed liquor. Studies by Shaw et al. (2006) indicated that high concentrations of NO₃⁻ can positively affect the emission of N₂O in a nitrifier denitrification process, making NO₃⁻ concentration one of the important variables that could be related to N₂O generation during denitrification in activated sludge systems.

Some N₂O emission parameters verified in this research are shown in Table 3. The average N₂O emission was 0.181, 0.058 and 0.016 gN₂O-N/cycle for S-I, S-II and S-III, respectively. In terms of volume of wastewater treated, the flow-based emission factors observed were 3.29·10⁻³, 1.05·10⁻³ and 0.29·10⁻³ gN₂O-L for S-I, S-II and S-III, respectively. Considering the volume of effluent treated in a cycle, and assuming a per capita wastewater generation of 160 L/person·d, the EF observed during S-I, S-II and S-III were 192.2, 61.6 and 17.0 gN₂O-N/person·year. These values are much higher than the guidelines proposed by the IPCC (2006), of 3.2 gN₂O/N/person·year (i.e., 2.04 gN₂O-N/person·year) in the case of wastewater treatment systems with controlled nitrification and denitrification. However, this EF proposed by the IPCC was based on a single experiment of Czepiel et al. (1995), performed in a WWTP in Durham, in northern United States (temperate climate). Therefore, the present study suggests that the N₂O emission by biological treatment systems located in subtropical/tropical regions is likely higher than the emission in temperate regions. Such higher EF values were also observed by Mello et al. (2013) in an intermittent aeration activated sludge system located in the highlands of Rio de Janeiro, Brazil, which is a subtropical region.

The total nitrogen removal, the fraction of nitrogen denitrified to N₂O and to N₂, and the total influent Nitrogen conversion to N₂O are shown in Table 4. The results show that most of the total denitrified nitrogen was converted to N₂O, while only a small fraction was converted to N₂. Although the complete denitrification process did not occur, N₂ generation predominated over N₂O generation in 84:16, 95:5, and 99:1 ratios for S-I, S-II and S-III, respectively. These results indicate that S-III, with the longest anoxic time, resulted in the lowest N₂O emission among the studied strategies, both in terms of emission factor and TN to N₂O conversion. The ratios of denitrified nitrogen emitted as N₂O observed in this study are within the range of values observed by Foley et al. (2010) in a study involving seven WWTPs in Australia. The authors reported a wide oscillation between the percentages of nitrogen denitrified to N₂O compared to the total nitrogen denitrified, ranging from 0.06 to 25.3%.

The fraction of TN converted to N₂O ranged from 0.47% in S-III to 5.28% in S-I. These values are below the value reported by Sun et al. (2013), who recorded a conversion of total nitrogen to N₂O of 6.52% in a full scale SBR. The same authors reported a conversion of 1.95% of the influent TN to N₂O in a real scale A²O system, this value being within the range observed in the present study. The conversion rate verified in S-II is also very similar to the conversion presented by Castro-Barros et al. (2015), who noted that 2.0% of the incoming nitrogen load was converted to N₂O.

Kong et al. (2013) analyzed the emission of N₂O by a biofilm SBR under intermittent aeration, with intentional nitrite accumulation to favor the anammox process. The fraction of influent nitrogen converted to N₂O was 1.50 ± 0.22%. This percentage of conversion is very close to what was observed in S-II, in which the occurrence of partial nitrification was also observed. By using molecular biology techniques, the authors found that *Nitrosospira* bacteria were the dominant gender of AOB responsible for N₂O emissions via nitrifier denitrification.

A review study done by Kampschreur et al. (2009) listed conversions of influent nitrogen to N₂O ranging from 0.001 to 14.6%. A wide variation of conversion to N₂O was also found in a national survey conducted by Ahn et al. (2010) in the United States, where conversions ranging from 0.01 to 1.8% of the influent nitrogen were observed. Although there are variations between the values obtained by different authors, it is noted that the total nitrogen fraction converted to N₂O tends to stay

### Table 3. N₂O emission parameters for S-I, S-II and S-III.

| Strategy | Emission per cycle (g N₂O-N/cycle) | Daily emission (g N₂O-N/d) | EF (g N₂O-N/person·year) | FBEF (g N₂O-N/L) |
|----------|-----------------------------------|-----------------------------|--------------------------|------------------|
| S-I      | 0.181                             | 1.086                       | 192.19                   | 3.29·10⁻³        |
| S-II     | 0.058                             | 0.348                       | 61.59                    | 1.05·10⁻³        |
| S-III    | 0.016                             | 0.064                       | 16.99                    | 0.29·10⁻³        |

### Table 4. Nitrogen removal, fraction of Nitrogen denitrified to N₂O and to N₂, and total influent Nitrogen conversion to N₂O.

| Strategy | TN removal (%) | Denitrification N₂O (%) | Denitrification N₂ (%) | Influent N conversion to N₂O (%) |
|----------|----------------|-------------------------|------------------------|----------------------------------|
| S-I      | 37.9           | 15.8                    | 84.2                   | 5.28                             |
| S-II     | 36.2           | 5.1                     | 94.9                   | 1.95                             |
| S-III    | 44.3           | 1.1                     | 98.9                   | 0.47                             |

*Brazilian Journal of Chemical Engineering*
Researching New Ways to Reduce N\textsubscript{2}O Emission from a Granular Sludge Sequencing Batch Reactor Treating Domestic Wastewater...

The N\textsubscript{2}O EF obtained in this research is superior to the EF proposed by the IPCC (2006), it is consistent with several studies reporting N\textsubscript{2}O emissions by activated sludge systems. Daelman et al. (2013), studying the N\textsubscript{2}O emission by a municipal activated sludge WWTP, reported an emission factor of 163.2 g N\textsubscript{2}O–N/person·year. This EF is 80 times higher than the EF proposed by the IPCC, and even much higher than the EF obtained in S-II and S-III. Mello et al. (2013), investigating the emission of N\textsubscript{2}O by an activated sludge WWTP with intermittent aeration, observed an EF of 8.76 g N\textsubscript{2}O/person·year, i.e., 5.57 g N\textsubscript{2}O–N/person·year. This EF is also higher than the EF proposed by the IPCC (2006), although it is lower than the values observed in the present research.

The average FBEF ranged from 3.29·10\textsuperscript{-3} gN\textsubscript{2}O/L in S-I to 0.29·10\textsuperscript{-3} gN\textsubscript{2}O/L in S-III. These values are much higher than the factor reported by Mello et al. (2013) of 8.0·10\textsuperscript{-4} g N\textsubscript{2}O/L, referring to an activated sludge system with intermittent aeration. However, this study was carried out in a region of humid subtropical climate, located at 600 meters of altitude, during the winter, unlike the conditions of the present study. In addition, the occurrence of nitrite accumulation in the system was not reported by the authors. These conditions may help to explain the low N\textsubscript{2}O emission verified by the authors, in relation to the values obtained in the present research.

Castro-Barros et al. (2015) studied the emission of N\textsubscript{2}O by a full-scale partial nitritation-anammox granular sludge reactor. The nitrogen load applied to the reactor was 1.75 kg NH\textsubscript{4}\textsuperscript{+}/N/m\textsuperscript{2}·d, this load being around 10 times higher than the load applied in the present research. The authors verified that the conversion of influent nitrogen to N\textsubscript{2}O presented an average value of 2.0%, very similar to the conversion observed in S-II, which corroborates the results verified in the present study. In spite of the wide variation between the applied nitrogen loads, the percentage of nitrogen converted to N\textsubscript{2}O was quite similar in both cases.

The nitrite accumulation that occurred in this study, as a result of partial nitrification, may have been one of the factors which favored N\textsubscript{2}O production during denitrification (Kampschreur et al., 2009). However, since nitrite accumulation was not directly related to N\textsubscript{2}O emission, there might be other factors influencing N\textsubscript{2}O emission. As noted by Quan et al. (2012), N\textsubscript{2}O emission could also be related to the granule constitution, since the spatial structure of the granules may induce incomplete denitrification, which may also lead to significant N\textsubscript{2}O generation.

The results obtained in the present study indicate that S-III, with the longest anoxic phase, promoted the lowest N\textsubscript{2}O emission and the highest ammonium removal rate among the studied strategies, probably due to a higher consumption by a better developed anoxic community. The emission factor and the conversion to N\textsubscript{2}O verified in S-III were 11 times lower than in S-I, and 4 times lower than in S-II.

**Microbial Communities**

The AGS composition was dominated by the genera *Pseudomonas* sp. (17%), *Comamonas* sp. (19%) and *Pseudoxanthomonas* sp. (45%) under S-I, S-II and S-III, respectively. The microbial dynamics characterized by new generation sequencing showed fluctuations along the operational strategies. Under S-I (64\textsuperscript{st} and 83\textsuperscript{rd} days), the most abundant families were Caulobacteraceae, Sphingomonadaceae, Pseudomonadaceae and Rhodocyclaceae. Under S-II (189\textsuperscript{th} day), the family Comamonadaceae was highlighted with relative abundance of 22%, whereas under S-III (293\textsuperscript{rd} and 391\textsuperscript{st} days), the Xanthomonadaceae predominated with 45%. A decrease in the relative abundance of Pseudomonadaceae (genus *Pseudomonas* sp.), as well as an increase of Xanthomonadaceae (genus *Pseudoxanthomonas* sp.) was observed over time during the studied strategies. It is important to point out that both populations are denitrifying. In addition, *Pseudoxanthomonas* sp. is a relevant community for granule structure, since they are EPS producers (Weissbrodt et al., 2014). This result corroborates the better stability of the system in terms of granular biomass characteristics obtained under S-III.

In terms of microorganisms related to the nitrogen cycle, DNA new generation sequencing underestimated *Nitrosomonas* and *Nitrospira* sequences, not detecting them. However, they were identified with FISH analysis. AOB hybridizing to probe Nso190 was identified in low abundance in S-I, which is in accordance with low ammonium oxidation activity at this period. Lower AOB activity could arise from competition with heterotrophic bacteria for oxygen (Okabe et al., 1999). In S-II and S-III, corresponding to the 189\textsuperscript{th} and 293\textsuperscript{rd} days, there was a gradual increase in the abundance of AOB, correlating with a higher nitrification rate. Hybridization with Ntspa662 probes was carried out to identify the presence and distribution of *Nitrospira* in the granular sludge. *Nitrospira* was present as small clusters in low abundance during all strategies. Since nitrite accumulated in the reactor at most times, it was directly available to NOB from the bulk liquid (Kim et al., 2006), but conditions (discussed above) were not completely favorable to their high proliferation.

Regarding denitrifiers, a positive hybridization signal was observed for *Pseudomonas* sp. in samples of S-I and S-III, indicating this genus as a feasible community for nitrite denitrification processes. However, the availability of sufficient organic carbon is the key...
factor in NO and N₂O consumption activities, as previously reported (Kampschreur et al., 2009). It can be concluded that the longer anoxic phase in the SBR operation cycle (S-III) promoted a BOD removal improvement by denitrifiers such as Pseudomonas, ensuring lower emissions of N₂O in the granular sludge reactor under these conditions.

CONCLUSIONS

The treatment performance and the N₂O emission from a pilot-scale SBR with AGS operated under three cycle configurations and fed with domestic wastewater under subtropical climate conditions were monitored and quantified. The nitrification process was incomplete, with nitrite accumulation occurring, which was mainly attributed to the temperature and to the cycle duration. There was a significant reduction of 91% both in the TN to N₂O conversion and in the EF verified for the studied strategies, which were associated with the extension of the SBR cycle anoxic phase and with the higher HRT. Furthermore, the anoxic phase extension and the HRT increase were also associated with higher BOD and ammonium removal rates and with a better biomass stability.

ACKNOWLEDGEMENTS

The authors would like to thank CNPq, the Pronex/FAPESC project and the Renutres/FINEP project, for their financial support, and the Pharmacotechnical Laboratory from Federal University of Santa Catarina, for the granulometry analyses.

REFERENCES

Ahn, J.H., Kim, S., Park, H., Rahm, B., Pagilla, K., and Chandran, K., N₂O emissions from activated sludge processes, 2008-2009: results of a national monitoring survey in the United States. Environ. Sci. Technol., 44(12), 4505-4511 (2010). https://doi.org/10.1021/es903845y

APHA – American Public Health Association, Standard methods for the examination of water and wastewater, 21st edition. Washington, DC (2005).

Castro-Barros, C.M., Daelman, M.R.J., Mampaey, K.E., Van Loosdrecht, M.C.M., and Volcke, E.I.P., Effect of aerations regime on N₂O emission from partial nitritation-anammox in a full-scale granular sludge reactor. Water Res., 68, 793-803 (2015). https://doi.org/10.1016/j.watres.2014.10.056

Coma, M., Verawaty, M., Pijuan, M., Yuan, Z., and Bond, P.L., Enhancing aerobic granulation for biological nutrient removal from domestic wastewater. Bioresour. Technol., 103(1), 101–108 (2012). https://doi.org/10.1016/j.biortech.2011.10.014

Czepiel, P., Crill, P., and Harriss, R., Nitrous oxide emissions from municipal wastewater treatment. Environ. Sci. Technol., 29(9), 2352-2356 (1995). https://doi.org/10.1021/es00009a030

Daelman, M.R.J., Van Voorthuizen, E.M., Van Dongen, L.G.J.M., Volcke, E.I.P., and Van Loosdrecht, M.C.M., Methane and nitrous oxide emissions from municipal wastewater treatment – results from a long-term study. Water Sci. Technol., 67.10, 2350-2355 (2013). https://doi.org/10.2166/wst.2013.109

De Kreuk, M.K., Heijnen, J.J., and Van Loosdrecht, M.C.M., Simultaneous COD, nitrogen and phosphate removal by aerobic granular sludge. Biotechnol. Bioeng., 90(6), 761-769 (2005). https://doi.org/10.1002/bit.20470

De Kreuk, M.K., Kishida, N., and Van Loosdrecht, M.C.M., Aerobic granular sludge – state of the art. Water Sci. Technol., 55(8–9), 75-81 (2007). https://doi.org/10.2166/wst.2007.244

Di Bella, G., Torregrossa, M., Simultaneous nitrogen and organic carbon removal in aerobic granular sludge reactors operated with high dissolved oxygen concentration. Bioresour. Technol., 142, 706-713 (2013). https://doi.org/10.1016/j.biortech.2013.05.060

Figueroa, M., Mosquera-Corral, A., Campos, J.L., and Méndez, R., Treatment of saline wastewater in SBR aerobic granular reactors. Water Sci. Technol., 58(2), 479-485 (2008). https://doi.org/10.2166/wst.2008.406

Foley, J., De Haas, D., Yuan, Z.G., Lant, P., Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. Water Res., 44, 831-844 (2010). https://doi.org/10.1016/j.watres.2009.10.033

Guimarães, L.B., Mezzari, M.P., Daudt, G.C., and Costa, R.H.R., Microbial pathways of nitrogen removal in aerobic granular sludge treating domestic wastewater. J. Chem. Technol. Biotechnol., 92, 1756-1765 (2017). https://doi.org/10.1002/jctb.5176

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., Van Loosdrecht, M.C.M., and Heijnen, J.J., The Sharon process: an innovative method for nitrogen removal from ammonium-rich waste water. Water Sci. Technol., 37(9), 135-142 (1998). https://doi.org/10.2166/wst.1998.0350

IPCC – Intergovernmental Panel on Climate Change, Guidelines for National Greenhouse Gas Inventories. Waste, Vol. 5, Kanagawa, Japan (2006).

Isanta, E., Suárez-Ojeda, M.E., Val del Río, Á., Morales, N., Pérez, J., and Carrera, J., Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater. Chem. Eng. J., 198-199, 163–170 (2012). https://doi.org/10.1016/j.cej.2012.05.066
Researching New Ways to Reduce N₂O Emission from a Granular Sludge Sequencing Batch Reactor Treating Domestic Wastewater...

Itohara, H., Hanaki, K., and Matsuo, T., Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. Water Res., 35(3), 657-664 (2001). https://doi.org/10.1016/S0043-1354(00)00309-2

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., Van Loosdrecht, M.C.M., Nitrous oxide emission during wastewater treatment. Water Res., 43(17), 4093-4103 (2009). https://doi.org/10.1016/j.watres.2009.03.001

Kampschreur, M.J., Van Der Star, W.R.L., Wielders, H.A., Mulder, J.W., Jetten, M.S.M., and Van Loosdrecht, M.C.M., Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment. Water Res., 42, 812-826 (2008). https://doi.org/10.1016/j.watres.2007.08.022

Kim, D.-J., Lee, D.-I., and Keller, J., Effect of temperature and free ammonia on nitrification and nitrite accumulation in landfill leachate and analysis of its nitrifying bacterial community by FISH. Bioresour. Technol., 97, 459–468 (2006). https://doi.org/10.1016/j.biortech.2005.03.032

Kong, Q., Zhang, J., Miao, M., Tian, L., Guo, N., and Liang, S., Partial nitrification and nitrous oxide emission in an intermittently aerated sequencing batch biofilm reactor. Chem. Eng. J., 217, 435-441 (2013). https://doi.org/10.1016/jcej.2012.10.093

Liu, Y., Kang, X., Li, X., and Yuan, Y., Performance of aerobic granular sludge in a sequencing batch bioreactor for slaughterhouse wastewater treatment. Bioresour. Technol., 190, 487-491 (2015). https://doi.org/10.1016/j.biortech.2015.03.008

Liu, Y., and Tay, J.H., State of the art of biogranulation technology for wastewater treatment. Biotechnol. Adv., 22, 533-563 (2004). https://doi.org/10.1016/j.biotechadv.2004.05.001

Liu, Y.-Q., Moy, B., Kong, Y.-H., and Tay, J.-H., Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzyme Microb. Technol., 46, 520-525 (2010). https://doi.org/10.1016/j.enzmictec.2010.02.001

Liu, Y.Q., and Tay, J.H., Influence of cycle time on kinetic behaviors of steady-state aerobic granules in sequencing batch reactors. Enzyme Microb. Technol., 41(4), 516–522 (2007). https://doi.org/10.1016/j.enzmictec.2007.04.005

Mannina, G., and Cosenza, A., Quantifying sensitivity and uncertainty analysis of a new mathematical model for the evaluation of greenhouse gas emissions from membrane bioreactors. J. Membr. Sci., 475, 80-90 (2015). https://doi.org/10.1016/j.memsci.2014.10.008

Mello, W.Z., Ribeiro, R.P., Broto, A.C., Kligerman, D.C., Piccoli, A.S., and Oliveira, J.L.M., Nitrous oxide emissions from an intermittent aeration activated sludge system of an urban wastewater treatment plant. Quim. Nova, 36(1), 16-20 (2013). https://doi.org/10.1590/S0100-40422013000100004

Moreira, I.S., Amorim, C.L., Ribeiro, A.R., Mesquita, R.B.R., Rangel, A.O.S.S., Van Loosdrecht, M.C.M., Tiritan, M.E., and Castro, P.M.L., Removal of fluoxetine and its effects in the performance of an aerobic granular sludge sequential batch reactor. J. Hazard. Mater., 287, 93-101 (2015). https://doi.org/10.1016/j.jhazmat.2015.01.020

Okabe, S., Satoh, H., and Watanabe, Y., In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. Appl. Environ. Microbiol., 65, 3182–3191 (1999).

Poot, V., Hoekstra, M., Geleijnse, M., Van Loosdrecht, M.C.M., and Pérez, J., Effects of the residual ammonium concentration on NOB repression during partial nitration with granular sludge. Water Res., 106, 518–530 (2016). https://doi.org/10.1016/j.watres.2016.10.028

Pronk, M., De Kreuk, M.K., De Bruin, B., Kamminga, P., Kleerebezem, R., and Van Loosdrecht, M.C.M., Full scale performance of the aerobic granular sludge process for sewage treatment. Water Res., 84, 207-217 (2015). https://doi.org/10.1016/j.watres.2015.07.011

Quan, X., Zhang, M., Lawlor, P.G., Yang, Z., and Zhan, X., Nitrous oxide emission and nutrient removal in aerobic granular sludge sequencing batch reactors. Water Res., 46, 4981-4990 (2012). https://doi.org/10.1016/j.watres.2012.06.031

Rathnayake, R.M.L.D., Song, Y., Tumendelger, A., Oshiki, M., Ishii, S., Satoh, H., Toyoda, S., Yoshida, N., and Okabe, S., Source identification of nitrous oxide on autotrophic partial nitrification in a granular sludge reactor. Water Res., 47, 7078-7086 (2013). https://DOI.ORG/10.1016/J.WATRES.2013.07.055

Schwarzenbeck, N., Erley, R., and Wilderer, P.A., Aerobic granular sludge in an SBR-sytem treating wastewater rich in particulate matter. Water Sci. Technol., 49(11-12), 41-46 (2004). https://doi.org/10.2166/wst.2004.0799

Shaw, L.J., Nicol, G.W., Smith, Z., Fear, J., Prosser, I.J., and Baggs, E.M., Nitrospira spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ. Microbiol., 8(2), 214-222 (2006). https://doi.org/10.1111/j.1462-2920.2005.00882.x

Sun, S., Cheng, X., Li, S., Qi, F., Liu, Y., and Sun, D., N₂O emission from full-scale urban wastewater treatment plants: a comparison between A/O and SBR. Water Sci. Technol., 67(9), 1887-1893 (2013). https://doi.org/10.2166/wst.2013.066
Van Loosdrecht, M.C.M., Jetten, M.S.M., Microbiological conversions on nitrogen removal. Water Sci. Technol., 38(1), 1-7 (1998). https://doi.org/10.2166/wst.1998.0002
Van Loosdrecht, M.C., Nielsen, P.H., Lopez-Vazquez, C.M., and Brdjanovic, D. (Eds.), Experimental Methods in Wastewater Treatment, Vol. 15. DOI: 10.2166/9781780404752, IWA Publishing, London (2016). https://doi.org/10.2166/9781780404752
Wagner, J., and Costa, R.H.R., Aerobic granulation in a sequencing batch reactor using real domestic wastewater. J. Environ. Eng., 139(11), 1391-1396 (2013). https://doi.org/10.1061/(ASCE)EE.1943-7870.0000760
Wang, F., Yang, F.L., Zhang, X.W., Liu, Y.H., Zhang, H.M., and Zhou, J., Effects of cycle time on properties of aerobic granules in sequencing batch airlift reactor. World J. Microbiol. Biotechnol. 21(8-9), 1379-1384 (2005). https://doi.org/10.1007/s11274-005-5451-2
Wang, S.G., Liu, X.W., Gong, W.X., Gao, B.Y., Zhang, D.H., and Yu, H.Q., Aerobic granulation with brewery wastewater in a sequencing batch reactor. Bioresour. Technol., 98, 2142-2147 (2007). https://doi.org/10.1016/j.biortech.2006.08.018
Wei, D., Shi, L., Zhang, G., Wang, Y., Shi, S., Wei, Q., and Du, B., Comparison of nitrous oxide emissions in partial nitrifying and full nitrifying granular sludge reactors treating ammonium-rich wastewater. Bioresour. Technol., 171, 487-490 (2014). https://doi.org/10.1016/j.biortech.2014.08.071
Weissbrodt, D.G., Shani, N., and Holliger, C., Linking bacterial population dynamics and nutrient removal in the granular sludge biofilm ecosystem engineered for wastewater treatment. FEMS Microbiol. Ecol., 88(3), 579–595 (2014). https://doi.org/10.1111/1574-6941.12326
Yang, J., Trela, J., Plaza, E., and Tjus, K., N₂O emissions from a one stage partial nitrification/anammox process in moving bed biofilm reactors. Water Sci. Technol., 68(1), 144-152 (2013). https://doi.org/10.2166/wst.2013.232