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Title

Evaluation of two start-up strategies to obtain nitrogen removal via nitrite and examination of the nitrous oxide emissions for different nitrification levels during the treatment of slaughterhouse wastewater

Short Title

Nitrogen removal via nitrite and examination of nitrous oxide emission

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Abstract

BACKGROUND: This study investigated the use of a novel start-up strategy to obtain nitrogen removal via nitrite using real-time aeration control, in a sequencing batch reactor (SBR₁), for the treatment of slaughterhouse wastewater. In addition, another SBR (SBR₂) was operated with the same objective, but with a start-up from seed sludge. Furthermore, analysis of the nitrous oxide (N₂O) production and emission was conducted for different levels of **nitritation degree** (ND).

RESULTS: Through the absence of inoculum in SBR₁, the nitrifying population could be regulated from the beginning and prevented nitrite oxidising bacteria to occur. This was proved by the ND, which was already above 80% from day 28. Although, it lasted 59 days to achieve a sufficient nitrogen removal efficiency (> 80%). In contrast for SBR₂, it took 90-110 days to achieve full nitrification, however the nitrogen removal efficiency was above 90% at all time. The maximum N₂O emission resulted in 0.12% of the nitrogen load.

CONCLUSION: This study suggested the importance of the seeding sludge for the achievement of full nitrification. Moreover, a strong positive relationship between the ND and N₂O emission was observed.

Keywords

NOB repression, aeration phase length control, slaughterhouse wastewater, SBR, nitrous oxide, nitrification

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1 **Introduction**

2 Regarding the food and beverage production, the meat processing industry turns out to be the
3 largest consumer of freshwater with a share of 24%, due to the slaughtering of animals and
4 cleaning of the slaughterhouse facilities¹. Because of these different activities, slaughterhouse
5 wastewater generally contains high levels of COD, nitrogen (N) and phosphorus (P).
6 Therefore, treatment of slaughterhouse wastewater typically consists of a primary physical-
7 chemical step by coagulation-flocculation followed by a biological nutrient removal step in an
8 activated sludge plant. As the primary treatment has removed large amounts of fat, oil, grease
9 and COD, the N concentrations remain moderate. As a result, COD may be limited for
10 complete nitrification-denitrification, leading potentially to nitrate discharge in the
11 environment.

12
13 A short-cut in the nitrogen cycle via the nitrite pathway reduces the oxygen demand in the
14 nitrification stage by 25% and the COD demand in the denitrification stage by 40%, resulting
15 in 20% less CO₂ emission and 30-50% less sludge production². Application of the innovative
16 nitrite pathway could significantly improve the performance of the biological treatment of
17 slaughterhouse wastewater. In recent years, different parameters have been proposed to obtain
18 a suppressed population of nitrite oxidising bacteria (NOB) or a promoted population of
19 ammonium oxidising bacteria (AOB); (1) low SRT (**1 – 8 days**) in combination with high
20 temperature (**25 – 35 °C**) as in the SHARON process³, (2) free ammonia (FA) and free nitrous
21 acid (FNA) inhibition², (3) low dissolved oxygen (DO) concentration (< 0,5 – 1,0 mg/l)⁴, (4)
22 alternating aeration pattern⁵; (5) specific inhibitors⁶ and (6) aeration phase length control^{7,8}.

23 Application of aeration phase length control proved to be a sustainable way to achieve nitrite
24 accumulation as it also optimizes the performance and efficiency of the biological nitrogen

removal plant^{9–11}. However, it was shown that NOB suppression through the use of aeration phase length control is a very slow process. Blackburne et al. (2008)⁷ reported more than 300 days, to achieve >80% nitrification, using a model based approach, Lemaire et al. (2008)¹⁰ and Dobbeleers et al. (2017)¹¹ reported >80% nitrification after 80 – 100 days using lab-scale SBR systems, inoculated with seed sludge, treating industrial wastewater from the food industry. Recently, Liu et al. (2017)¹² achieved a very high nitrification, >90%, in only 23 days through the use of an aerobic starvation period of 14 days and a reactivation period of 9 days.

Regardless of the benefits of N removal via nitrification-denitrification, it was shown that high nitrite accumulation levels act as precursor for nitrous oxide (N₂O) production^{13,14}. N₂O is an important greenhouse gas with a 300-fold stronger effect as CO₂, and contributes to the destruction of the stratospheric ozone layer¹⁵. Three pathways, during biological nitrogen removal, are generally recognized as main source of biological N₂O formation. During (1) hydroxylamine oxidation, N₂O is formed as a by-product during the oxidation of hydroxylamine to nitrite^{16,17}. Under low oxygen concentration, (2) nitrifier denitrification will be stimulated^{14,17}, as autotrophic AOB will reduce nitrite to N₂O. AOB do not have N₂O reductase, resulting in N₂O as the end product of the nitrifier denitrification pathway¹⁶. Furthermore, it has been shown that during (3) heterotrophic denitrification, N₂O will be an obligate intermediate in the reduction of nitrite to N₂¹⁴.

Rodriguez-Caballero et al. (2013)¹⁸ reported an N₂O emission of 1.2% of the converted N using a lab-scale nitrification SBR treating artificial wastewater. Kampschreur et al. (2008)¹³ determined N₂O emission of a full-scale nitrification reactor and found an emission of 1.7% of the N load. In contrast, Desloover et al. (2011)¹⁹ reported N₂O emissions up to 6.6% of the nitrogen load, while investigating a full-scale nitrification reactor, using anaerobically pre-treated wastewater. Recently, Dobbeleers et al. (2017)¹¹ examined a lab-scale SBR treating anaerobic pre-treated wastewater originating from the potato industry. They found N₂O

emissions between 3.6 – 4.0 % of the N-converted, using nitrification-denitrification through the use of aeration phase length control. Although considerable research, regarding N₂O emissions, has been conducted on artificial and municipal wastewater²⁰, less attention has been paid to nitrification reactors treating industrial wastewater such as slaughterhouse wastewater.

In current study, we investigate a novel start-up strategy based on preventing NOB occurrence from the start, instead of trying to repress the existing NOB population. Therefore, an SBR was first started-up without inoculum, and subsequently operated using real-time aeration phase length control in a step-feed configuration. To evaluate this novel start-up strategy, a second SBR was started-up with inoculum. Additionally, the production and emissions of N₂O during different levels of nitrite accumulation were determined.

Material and Methods

Reactor set-up

Two lab-scale SBR's, SBR₁ and SBR₂, with an internal diameter of 230 mm, maximum height of 400 mm and working volume of 12L were used in this study (H/D = 1.74). Feeding was performed by an electromagnetic IWAKI[®] ES pump (Tessenderlo, Belgium). Effluent withdrawal was accomplished by an electric ER-PLUS[®] valve (Eriks, Hoboken, Belgium) which was located on a height of 220 mm. A heidolph[®] RZR 2020 (Schwabach, Germany) mechanic mixer was used to maintain the sludge in suspension. An air pump (Rena, Annecy, France) connected to a ceramic air diffuser provided sufficient oxygen distribution through the mixed liquor.

The SBR sequence and performance of all hardware actions (mixer, air pump, feeding pump and discharge valve) were controlled by a Siemens[®] programmable logic controller (PLC)

(Beersel, Belgium) which could be manipulated by a custom-build LabVIEW (National Instruments, Texas, USA) supervision program. Additionally, DO, pH and oxidation reduction potential (ORP) (Hach, Mechelen, Belgium) were continuously measured and visualised by the LabVIEW supervision program.

Both SBR's were operated at ambient temperature (18-22°C). Figure 1 shows a schematic overview of the applied cycle operation, which was based on preliminary experiments, for both SBR's. During each cycle, 2L of slaughterhouse wastewater was pumped in the reactor in 3 equal feeding periods (20 min). Each anoxic feeding period was followed by an anoxic step (50 min) and an aeration step with variable duration (20 – 50 min, see below). To end the cycle, an endogenous anoxic phase (120 min), a refreshment phase (5 min), settling (120 min) and effluent withdrawal (15 min) were executed. During the aerobic steps, the DO was controlled between 0.5 and 1.5 mg O₂/L, using an on/off regulation.

The key difference between both reactors was the start-up of each SBR. To start up SBR₁, the reactor was filled with slaughterhouse wastewater, 940 mg soluble COD (SCOD)/L and 110 mg NH₄-N/L, and no inoculum was used. During the first 10 days, aeration steps of 5h were alternated with non-aeration periods of 1.5h to stimulate initial sludge growth. Moreover, no additional feeding was performed during this initial 10 days. Subsequently, the SBR was operated as described above (Figure 1).

In contrast, SBR₂ was filled with inoculum (3.7 gVSS/L) originating, from the full-scale SBR at the slaughterhouse and operated as described above (Figure 1). In order to examine, nitritation stability, after 226 days, the variable aeration steps of SBR₂ were changed to fixed aeration steps with a duration of 50 min.

The SRT was kept at 20 days for both reactors, by periodically removing the excess sludge from the SBR's during the post-anoxic phase.

Wastewater

Pre-treated wastewater was sampled regularly from the slaughterhouse buffer tank and, after transportation, immediately stored at 4°C. The pre-treatment involves an enhanced dissolved air flotation (DAF), through the dosage of Fe_3Cl . The DAF COD removal efficiency is about 50-80%. Furthermore, part of the rainwater is also drained towards this buffer tank. The combination of this rainwater dilution effect and the alternating activities at the slaughterhouse (slaughtering and cleaning) resulted in a strong variable wastewater. During this study, both SBR's were fed with different batches of wastewater. The average composition of these batches is summarised in table 1.

Real-time control strategy

Due to an on-off aeration control during aerobic steps, the oxygen uptake rates (OUR) could be calculated from decreasing DO values. This online OUR calculation was performed automatically by the custom-build LabVIEW program. Furthermore, an adjustable OUR threshold value could be set, which was adjusted when necessary. It was shown that a sharp drop in OUR occurs as soon as the oxidation of $\text{NH}_4\text{-N}$ is (almost) complete¹⁰. Consequently, if the custom-build LabVIEW program detects an OUR value which is below the threshold value, it will switch immediately to the next step in the SBR cycle (for denitrification). In order to build in some prudence in the control strategy, aeration steps always had a minimum (20 minutes) and maximum duration (50 minutes).

Analyses

Specific AOB and NOB activity rates (SR_{AOB} , SR_{NOB}) were determined, using an offline respirometric assay as described by Dobbeleers et al. (2017)¹¹. Briefly, a custom-build respirometer was used to determine the specific nitrogen removal rates (SR) of both AOB, SR_{AOB} , and NOB, SR_{NOB} . In-situ cycle measurements during an aerobic step were conducted at least once a week. During this measurements, grab samples, at the start and at the end of an aeration step were analysed to provide profiles of NH_4 -N, NO_2 -N and NO_3 -N. These results allow the calculation of the nitrification degree (ND).

$$ND (\%) = \frac{[NO_2^- - N]_{t_0}^{t_n}}{[NO_2^- - N]_{t_0}^{t_n} + [NO_3^- - N]_{t_0}^{t_n}} \cdot 100$$

With t_0 , the start of the aeration step and t_n , the end of the aeration step.

All samples were filtered over a glass microfiber filter (particle retention 1.2 μ m). Concentrations of NH_4 -N, NO_2 -N, NO_3 -N and (S)COD were analysed using standard cuvette tests (Hanna Instruments, Temse, Belgium). Biomass concentrations (ML(V)SS) measurements were performed according to the standard methods²¹. N_2O production in the mixed liquor was monitored using a Unisense A/S (Aarhus, Denmark) microsensor with data logging. Before each measurement, a two-point calibration, as described by the instructions of Unisense was carried out.

Results

Performance of the SCOD and N-removal for both SBR's

During the first 10 days, the operation of SBR₁ (start-up from wastewater) involved alternating conditions of 5h of aeration with 1.5h of non-aeration. This resulted in a SCOD removal efficiency of 50% after 4 days, which improved towards 80% after 11 days. In contrast, no removal of N nor nitrite accumulation occurred during this start-up period.

Starting from day 11, the operational strategy was changed as discussed above. The first indication of N-removal occurred after 18 days, however, it took 25 days to achieve an N-removal efficiency of 32% and 59 days to realize 84% removal (figure 2A). The VSS concentration increased from 0.30 g/L on day 11 to 1.05 g/L on day 59, indicating significant sludge growth during this period. During the following 270 days, as many as 5 different influent batches with variable composition (table 1; figure 2A), were fed to the reactor. A high average SCOD and N-removal efficiency of respectively 93.6% and 94.2% could be maintained during this period.

SBR₂ (start-up from sludge) was operated for 240 days using the same operational strategy as SBR₁. Due to the start-up with sludge, the real-time control strategy could be applied immediately from day 1. Figure 2B, shows the SCOD and N-removal performance of SBR₂. Due to the use of adapted seed sludge originating from the slaughterhouse, proficient SCOD and N-removal were obtained instantly. During the entire operational period the average SCOD and N-removal were 96.3% and 95.7% respectively. Similar as for SBR₁, this high removal efficiencies could be maintained even though highly varying influent batches were fed to the reactor (table 1; figure 2B). As the aeration control strategies provided a dynamic aeration time, this is also reflected in a varying HRT (table 2). Moreover, the nitrogen and COD loading rates are determined by the dynamic aeration control as well (table 2).

Evaluation of the Shortcut Nitrification – Denitrification via nitrite

During standard reactor operation, the nitrification performance was frequently examined in both SBR's. 2 different methods were used to evaluate nitrogen removal via nitrite. The first method concerns the ND (see material and methods for equation), which was obtained through the analysis of the different N-compounds (NH₄-N, NO₂-N, NO₃-N) during an aeration phase. The second method concerns the ratio of the N-removal rates of AOB and

NOB (SR_{AOB} , SR_{NOB}). Figure 3 presents the results of these 2 methods for both SBR's. According to SBR₁, it can be observed that the ND was already >80% at day 28, and remained this high during the entire reactor operation. Due to the fact that sludge growth had to be stimulated during the reactor start-up, the earliest offline activity measurement, to determine SR_{AOB} and SR_{NOB} , was carried out on day 77. This resulted in a NOB/AOB activity ratio of 11%. The corresponding ND was 90% (figure 3A). During the further progress of SBR₁, the NOB/AOB activity ratio could always be maintained below 25%, the average SR_{NOB} was only 0.31 mg N/gVSS.h whereas the average SR_{AOB} was 4.21 mg N/gVSS.h. These results show the high level of nitrification obtained in SBR₁ and prove the effectiveness of **this innovative start-up strategy to obtain short-cut nitrification**. Without inoculum, a high level of N-removal via nitrite was obtained after only 59 days.

Two days after the inoculation of SBR₂, the first online cycle measurement was conducted, which resulted in an ND of 0.0%. Although no nitrite accumulation could be measured at the end of the aeration step, some minor nitrite accumulation was reported halfway the aeration step (data not shown). Furthermore, this temporary nitrite accumulation could be verified by the NOB/AOB activity ratio, which was 57%. During the next 5 to 10 days, the ND increased to 20 – 40%, but the NOB/AOB ratio remained unchanged (figure 3B). A breakthrough concerning the nitrification level took place between day 80 and 110, as the ND increased from 22% at day 81 to 82% at day 103. Correspondingly, the NOB/AOB activity ratio decreased from 43% at day 79 to 20% at day 107. During the following 110 days the high rate of nitrification could be maintained as the average ND was 82% and the average NOB/AOB activity ratio was 25%. In order to examine the nitrification stability, the real-time control of the aeration steps was switched to aeration steps with fixed duration of 50 minutes during the last 20 days. Due to this manipulation, the ND rapidly decreased to 9% after only 4 days.

1 However, a temporary nitrite accumulation of 35% (data not shown) halfway an aeration step
2 could still be observed until 15 days after the modification. Due to the extended aeration time,
3 NOB were able to oxidise the accumulated nitrite to nitrate, resulting in energy which they
4 could use for their growth. The reactivation of the NOB is clearly visible in figure 3B, as the
5 NOB/AOB ratio increased to 96% at day 240, meaning the conversion rates (SR) of NOB and
6 AOB are almost equal.

7 *Influence of the Nitrification Level on the Nitrous Oxide Emissions*

8 During the operation of SBR₂, nitrous oxide production was measured while in-situ online
9 cycle measurements were performed on different moments with varying ND values. This
10 allowed the analysis of the overall N mass balance. Figure 4 shows 2 extreme situations, (1) a
11 ND of 0% corresponding to a N₂O emission of 0.07%; (2) a ND of 85.5% corresponding to a
12 N₂O emission of 3.29%. Due to the high solubility of N₂O in water, emissions were only
13 calculated when the aeration pump was switched on¹¹. Figure 4 shows the estimated total
14 emission caused by each aeration event. It can be observed clearly that, due to the higher ND
15 or nitrite accumulation, the produced and emitted N₂O significantly increased. Furthermore,
16 for different levels of ND, corresponding N₂O emissions were obtained (Supplementary
17 Figure S1). A clear relationship between ND and N₂O emission can be observed from Figure
18 5.

23 **Discussion**

24 *Effect of the Start-up Strategy to obtain Nitrogen Removal via Nitrite*

In this study two different start-up strategies, to obtain nitrogen removal via nitrite were examined. It was shown that SBR₁ (without inoculum) resulted in a significantly faster achievement, (after 59 days) of sufficient nitrogen removal via nitrite. Although, SBR₂ (with inoculum) needed approximately 100 days to obtain an appropriate nitrification level, the N-removal efficiency was above 85% at all time. These results indicates, that depending of the application either of these start-up strategies might be preferable. Although, various studies about achieving nitrogen removal via nitrite through the use of aeration control strategies were carried out, most of them concern domestic wastewater^{7,22,23}. The reported start-up time to achieve a sufficient level of nitrite accumulation varies from approximately 40 days²² to more than 300 days⁷ (table 3). When starting up from seed sludge as SBR₁ in present study, Lemaire et al. (2008)¹⁰ achieved full nitrification after 80 to 100 days, using slaughterhouse wastewater. Moreover, Lemaire et al. (2008)¹⁰, suggest that through the use of real-time aeration control, which strictly controls the aeration step, NOB are gradually eliminated through the absence of their energy source (oxygen).

The importance of seed sludge, in order to achieve nitrification, cannot be underestimated. The presence and activity of both nitrifier groups, AOB and NOB, depends on the history and origin of the seed sludge, which directly influences the time needed to achieve an abundant level of nitrification. Blackburne et al. (2008)⁷ and Lemaire et al. (2008)¹⁰ also found that when the NOB conversion rates are higher than those of AOB, the time needed to achieve nitrification could be substantially longer. In present study, through the absence of seeding sludge and the employment of real-time aeration control (SBR₁), the microbial population could be controlled from the beginning. Since nitrification is a 2-step process, AOB were the first the occur as ammonia was the only substrate initially present in the wastewater. The application of the real-time control strategy, prevented NOB to utilize the produced nitrite, which proves nitrogen removal via nitrite as soon as a sufficient N-removal efficiency is obtained. The start-

up time of 59 days might be improved, if the aeration steps during the first 10 to 20 days would last longer than the applied maximum time of 50 minutes. However, as nitrite starts to accumulate, NOB growth is always around the corner. Recently, Liu et al. (2017)¹² achieved a nitrite accumulation level > 95% after 25 days using an aerobic starvation of 14 days to inhibit NOB and a reactivation period of 9 days to stimulate AOB. Moreover, they proved that strict control of the SRT, 15 days at 29 °C, was absolutely essential to maintain a high nitrite accumulation level. In our study, the SRT was 20 days at an average temperature of 20°C, which points to the aeration control strategy as the key to outcompete NOB. Similarly as in our study, Liu et al. (2017)¹² essentially showed that AOB are the first to appear, with ammonia as sole substrate, and that through the use of a control strategy, NOB growth could be prevented. Inhibition of NOB at higher temperature (25-35°C) in combination with SRT control has been studied previously^{2,25} as well as the effect of FA²⁶ and low DO⁴. Table 3 summarizes these different start-up strategies to achieve an NOB repressed system. As it can be observed, they all used seed sludge to inoculate there systems.

Influence of the Nitrification-Degree on the Production and Emission of Nitrous Oxide

In this study, N₂O production and emission were evaluated for different levels of ND. As shown in figure 4, a strong positive correlation between the N₂O emission and the ND was demonstrated. These findings can be attributed to the nitrifier denitrification pathway in which AOB reduce nitrite to N₂O²⁷. It has been reported by Wunderlin et al. (2012)¹⁴, that nitrifier denitrification is the most dominant N₂O formation pathway under aerobic conditions and is stimulated by elevated nitrite levels²⁷.

1 This study showed that, for an ND of 0%, and thus conventional 2-step nitrification an
2 negligible amount of 0.07 % $\text{N}_2\text{O-N}/\text{NH}_4\text{-N}$ consumed, was emitted. Contrary, for an ND
3 >80%, and thus the presence of elevated nitrite levels, more than 3.0 % $\text{N}_2\text{O-N}/\text{NH}_4\text{-N}$
4 consumed was emitted, proving the effect of elevated nitrite concentrations on the nitrifier
5 denitrification pathway. However, the obtained emission value is lower than reported in
6 several other studies using a lab-scale nitrification reactor^{28,29}. In contrast, Rodriguez-Caballero
7 et al. (2013)¹⁶, measured only 1.22 % $\text{N}_2\text{O-N}/\text{NH}_4\text{-N}$ consumed. The Intergovernmental Panel
8 on Climate Change (IPCC) applies a value of 0.5% of the nitrogen load as an estimate for
9 N_2O emission from wastewater¹³. Expressing our maximum emission data as a percentage of
10 the nitrogen load results in an emission value of 0.12%. This is significantly lower than
11 reported by other studies investigating partial nitrification^{13,17}.

12 Our study showed, that under persistent aerobic conditions, the N_2O production rate increased
13 substantially (Figure 4). These findings were also reported by several other studies^{30,31}. They
14 all propose a high switching frequency between aeration and anoxic phases (5 to 30 min), as
15 mitigation strategy for N_2O emissions. Alternating short aeration steps with short anoxic steps
16 prevents high accumulation of intermediaries as NO_2^{-30} . Although, in our study, aeration is
17 controlled by an on/off regulator, between 0.5 and 1.5 mg O_2/L , and a duration between 20
18 minutes and 50 minutes which still allows a frequently alternating aerated/anoxic system. This
19 specific aeration pattern might explain the lower N_2O emissions, however it might be
20 interesting to investigate the influence of the specific on/off aeration control on the N_2O
21 production and emission. Likewise, Rodriguez-Caballero et al. (2013)¹⁶ regulated the DO in
22 their lab-scale SBR system using an on/off PLC controller whereafter relatively low N_2O
23 emissions were obtained at full nitrification. However, further investigations about the aeration
24 pattern and DO regulation should be carried out in order to clarify the effect on the N_2O
25 emissions for distinct industrial wastewaters as slaughterhouse wastewater.

Conclusion

In this study, two start-up strategies to achieve nitrogen removal via nitrite through the use of real-time aeration control, were evaluated. It was shown that through the use of the innovative start-up without seed sludge the nitrifying population could be kept under control from the beginning rather than manipulating an existing microbial population when starting from seed sludge. Furthermore, it was shown that N_2O emission is directly correlated to the ND, caused by elevated nitrite levels which enhances nitrifier denitrification. Although, there is high probability that through the use of a frequent alternating aeration pattern, reduced N_2O emissions were obtained.

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Table 1. Influent compositions for the different batches in terms of (S)COD and NH₄-N for SBR₁ and SBR₂

Batch	Period (days)	Average $\pm$ SD		
		COD	SCOD	NH ₄ -N (mg/L)
		(mg/L)	(mg/L)	
SBR ₁ start-up <i>without</i> inoculum				
1	11-83	968 $\pm$ 115	816 $\pm$ 150	138 $\pm$ 19
2	84-167	1579 $\pm$ 81	1359 $\pm$ 115	171 $\pm$ 22
3	168 - 230	1488 $\pm$ 100	1361 $\pm$ 138	187 $\pm$ 33
4	231 - 265	2646	2202 $\pm$ 370	304 $\pm$ 57
5	266 - 331	901 $\pm$ 27	785 $\pm$ 117	98 $\pm$ 21
SBR ₂ start-up <i>with</i> inoculum				
1	1-25	987 $\pm$ 39	858 $\pm$ 32	85 $\pm$ 11
2	26-64	1847 $\pm$ 138	1513 $\pm$ 82	147 $\pm$ 29
3	64-113	819 $\pm$ 77	743 $\pm$ 45	95 $\pm$ 18
4	114-141	1171 $\pm$ 86	984 $\pm$ 167	77 $\pm$ 28
5	142-183	1083 $\pm$ 203	844 $\pm$ 115	119 $\pm$ 11
6	184-239	1025 $\pm$ 76	859 $\pm$ 65	142 $\pm$ 18

- 1 **Table 2.** Overview of the HRT(d), NH₄-N and COD loading rate (kg/m³.day) for the different SBR's.

	start-up from wastewater (SBR ₁)	start-up from sludge (SBR ₂)
HRT (d)	10.0* - 2.91	3.0-2.0
NH₄-N-loading rate (kg N/m³.day)	0.03-0.10	0.03-0.08
COD-loading rate (kg COD/m³.day)	0.05-0.85	0.32-0.65

- 2 *During start-up, the reactor was filled with wastewater and no additional feeding was added during the first 10 days.

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1 **Table 3.** Overview of different SBR start-up strategies to obtain nitrogen removal via nitrite

study	NOB repression aim	inoculum from	strategy	mechanism	nitrification start-up time (days)	wastewater	reactor type
Blackburne et al. (2008) ⁷	nitrification-denitrification	model based study	aeration control	suppress NOB	+300	domestic	model based study
Yang et al. (2007) ²²	nitrification-denitrification	full-scale WWTP	aeration control based on pH	suppress and washout of NOB	±66	domestic	pilot-scale SBR
Gu et al. (2012) ³²	partial nitrification	full-scale municipal WWTP	aeration control, DO and pH	suppress and washout of NOB	40	domestic	pilot-scale SBR
Lemaire et al. (2008) ¹⁰	nitrification-denitrification	lab-scale SBR	aeration control, DO and pH	suppress and washout of NOB	100	abattoir	lab-scale SBR
Blackburne et al. (2008) ⁴	partial nitrification	full-scale municipal WWTP	DO control (0.4 mg O ₂ /L)	outcompete NOB	-	synthetic	lab-scale CSTR
Katsou et al. (2015) ³⁴	nitrification-denitrification	full-scale WWTP	DO control (0.7 mg/L) and FA inhibition	outcompete NOB	±100	UASB effluent	lab-scale SBR
This study	nitrification-denitrification	none	OUR based aeration control	prevent NOB growth	59*	slaughterhouse	lab-scale SBR
This study	nitrification-denitrification	full-scale SBR	OUR based aeration control	suppress and washout of NOB	90-100	slaughterhouse	lab-scale SBR
Dobbeleers et al. (2017) ¹¹	nitrification-denitrification	full-scale industrial CSTR	OUR based control strategy	suppress and washout of NOB	80	UASB effluent of potato-processing industry	lab-scale SBR
Mulder et al. (1998) ³³	nitrification-denitrification	waste activated sludge from WWTP	temperature control (30-40°C)	outcompete NOB	-	domestic	full-scale reject water
Peng et al. (2004) ²⁶	nitrification-denitrification	-	FA inhibition	inhibit NOB	-	pharmaceutical	lab-scale SBR
Liu et al. (2017) ¹²	partial nitrification	lab-scale pilot reactor	aerobic starvation and SRT control	suppress NOB and control them during	23 (14 days starvation; 9 days reactivation)	domestic	lab-scale SBR

during recovery
reactivation
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3 **Figure 1. Schematic overview of the SBR cycle operation.**

4 Figure 2. Performance of SBR₁, start-up from wastewater (A) and SBR₂, start-up from sludge (B).

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6 NO₂-N (mg/L) and NO₃-N (mg/L) and removal efficiencies of SCOD (%) and N (%). The vertical
7 dotted lines represent a new batch of wastewater.

8 Figure 3. Evolution of the ND degree (%), NOB/AOB activity ratio (%), SR_{NOB} (mg N/gVSS.h) and
9 SR_{AOB} (mg N/gVSS.h), for SBR₁, start-up from wastewater (A) and SBR₂, start-up from sludge (B).

10 Figure 4. Online cycle measurements of SBR₂ displaying profiles of N₂O (μmol/L), DO (mg/L), NH₄-
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13 N/NH₄-N consumed (%). No nitrification occurring (ND = 0.0%) (A); High degree of nitrification (ND=
14 85.5%) (B).

15 Figure 5. Correlation between ND degree (%) and N₂O-N emission per NH₄-N consumed (%).

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4 N/gVSS.h). (Error bars = SD)

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Figure 4. Online cycle measurements of SBR₂ displaying profiles of N₂O (μmol/L), DO (mg/L), NH₄-N (mg/L), NO₂-N (mg/L) and NO₃-N (mg/L). Extrapolation of the linear N₂O formation (μmol/L) during the off aeration events was used to calculate the emitted N₂O (μmol/L) and cumulative N₂O-N/NH₄-N consumed (%). No nitritation occurring (ND = 0.0%) (**A**); High degree of nitritation (ND= 85.5%) (**B**).

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2 **Figure 5.** Correlation between ND degree (%) and N₂O-N emission per NH₄-N consumed (%).

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