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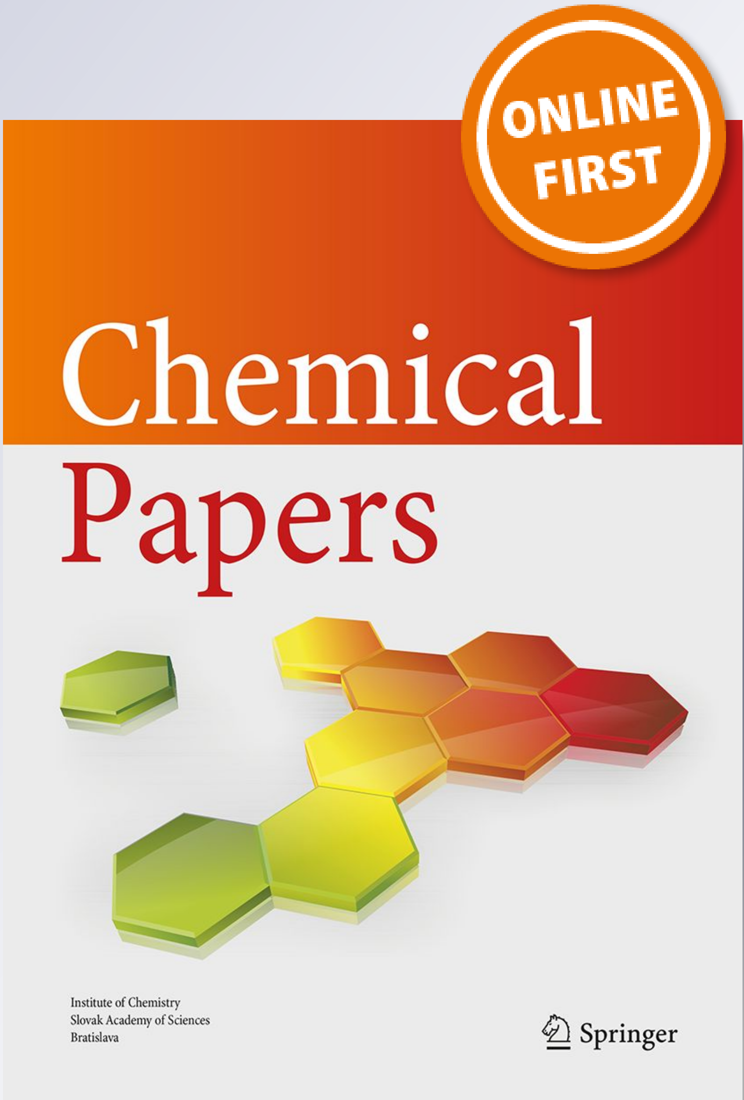

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ORIGINAL PAPER

High-performance system for partial nitritation of reject water resistant to temperature fluctuation

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Abstract A sequencing batch reactor (SBR) applying partial nitritation for reject water treatment was operated for 330 days at a laboratory scale. The system was repeatedly exposed to sudden temperature drops from 24 to 17 °C. The nitrogen loading rate (NLR) was increased incrementally from 0.4 to 1.5 kg/(m^3 day) with the aim to evaluate temperature stability of the process at different NLR value. Total nitrite nitrogen ($TN^{III}N$) represented 94–99% of oxidised nitrogen in the effluent throughout the entire operation of the reactor. It was found that the pH profile during the SBR cycle, nitrogen removal efficiency and concentration of N-species in the effluent did not show significant changes following temperature decreases occurring within the entire applied range of the NLR. Simultaneously, the nitrogen removal rate increased proportionally with the NLR where the nitrogen oxidation efficiency reached 48–58% regardless of actual temperature and NLR. These observations clearly demonstrate the temperature stability of applied partial nitritation system during the tested temperature fluctuations.

Keywords Nitrite accumulation · Sequencing batch reactor · Partial nitritation · Temperature fluctuation

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Introduction

Nitritation/denitritation and partial nitritation/anaerobic ammonium oxidation (ANAMMOX), have been frequently applied to reject water treatment in laboratories, pilot plants and full-scale conditions within the past few decades (Hellinga et al. 1998; van Dongen et al. 2001; van Kempen et al. 2001; Jenicek et al. 2004; Zekker et al. 2012; Lackner et al. 2014; Torà et al. 2014). The nitritation applied as the first stage for subsequent ANAMMOX process should be operated with the aim to convert partially total ammonia nitrogen (the sume of N-NH_4^+ and N-NH_3, TAN) to total nitrite (trivalent) nitrogen (the sum of N-NO_2^- and N-HNO_2, $TN^{III}N$) where suitable ratio of $TN^{III}N$/TAN concentration enabling satisfactory course of ANAMMOX process should be achieved (van Dongen et al. 2001; Zekker et al. 2012). Therefore, the term "partial nitritation" was established for these cases. Nitritation/denitritation as well as partial nitritation/ANAMMOX is based on the accumulation of nitrites induced by the selective inhibition of nitrite oxidising bacteria (NOB) during the nitrification process, whereby ammonia oxidising bacteria (AOB) activity is preserved. The main advantages of the above-mentioned processes include high effectiveness and the reduced costs of aerating and organic substrates, as compared to a traditional nitrification/denitrification system (Turk and Mavinic 1989; Mulder et al. 1995). Suppression of NOB activity can be achieved by a dissolved oxygen (DO) limitation (Ruiz et al. 2003; Ge et al. 2014; Pacek et al. 2015) or by the application of a short sludge retention time (SRT) at high temperature (Hellinga et al. 1998). Nitritation as well as partial nitritation during reject water treatment may be successfully achieved in a sequencing batch reactor (SBR) thanks to strong fluctuations in free ammonia (FA) and free nitrous acid (FNA) concentrations (Jenicek et al. 2004, Park and Bae 2009; Svehla et al. 2014). These chemical substances selectively inhibit

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NOB activity within a specific concentration range (Anthonisen et al. 1976; Vadivelu et al. 2007; Blackburne et al. 2008; Pambrun et al. 2008).

Temperature is an important factor affecting the nitrification process in a number of ways. Nitrifying organisms are generally highly sensitive to temperature. Thus, the productivity of nitrification (and partial nitritation as well) is strongly temperature-dependent. On the other hand, also the possibility to operate satisfactory biological systems applying nitrification at low temperature was proved in the past (Daija et al. 2016). Higher temperatures support the accumulation of nitrite during the nitrification process under specific conditions (Hellinga et al. 1998; Hao et al. 2002; Kim et al. 2008). At the same time, the temperature value significantly affects the distribution of FA and FNA (Park and Bae 2009; Anthonisen et al. 1976). Therefore, a change in temperature may influence the inhibition of nitrifying bacteria during high nitrogen loaded wastewater treatment, even at stable TAN and $TN^{III}N$ concentrations. The combined effect of high temperatures and SRT limitation is the principle of the Single reactor system for High activity Ammonium Removal Over Nitrite (SHARON) process (Hellinga et al. 1998). Current systems for separate biological treatment of reject water applying nitritation/denitritation, or partial nitritation/ANAMMOX are usually operated at 30–40 °C (Hellinga et al. 1998; Lackner et al. 2014; Volcke et al. 2006). Potential temperature fluctuations may affect the stability of the partial nitritation process due to the relatively high temperature sensitivity of AOB (Henze et al. 2008; Hrncirova et al. 2017), but heating the reactor significantly deteriorates the economy of the process. Therefore, it seems appropriate to look for technological alternatives that do not include temperature control (Rodríguez et al. 2011; Hu et al. 2013). Positively, partial nitritation with flocculent biomass at temperatures of 23 ± 2 °C was successfully operated during experiments performed by Svehla et al. (2014) indicating the possibility of applying lower temperatures for this process under specific conditions. Relatively high temperatures are typical for reject water arising during the thickening and dewatering of anaerobically digested sludge thanks to mesophilic or thermophilic conditions prevailing in the digesters. However, from a technological point of view, it may still be difficult to achieve temperatures exceeding 20 °C, for example, during the winter season in many regions. Additionally, actual temperature of raw reject water is influenced also by dewatering unit applied within the sludge management of particular wastewater treatment plant. For example, belt press with high portion of flush water produces colder reject water than centrifuge. Kouba et al. (2014) demonstrated that wastewater containing TAN in concentrations of up to 600 mg/L may be satisfactorily treated by partial nitritation in a SBR using biomass cultivated in the form of biofilm at 15 ± 1 °C and at a nitrogen loading rate (NLR) reaching 0.2 kg/(m^3 day). Svehla et al. (2015) confirmed that also the biomass cultivated in the form of activated sludge is able to ensure satisfactory operation of partial nitritation under conditions identical with Kouba et al. (2014). However, the biomass was exposed to low temperatures for a long time and the potential effect of large temperature fluctuations was not evaluated. In addition, the ability of the system to be operated at a higher NLR was not studied. Persson et al. (2014) confirmed the possibility of applying the partial nitritation/ANAMMOX process to reject water treatment in a moving bed biological reactor (MBBR) at even lower temperatures. In their experiment, the temperature was decreased from 19 to 10 °C in three steps. The NLR was reduced during the operation of the reactor in order to achieve satisfactory TAN removal efficiency. The system was stable within the temperature range of 19 and 13 °C. At 10 °C, unstable TAN removal was registered. Different authors have applied nitritation or partial nitritation without temperature control, reaching certain ranges of operational temperature depending on the ambient temperature during the treatment of reject water or other types of wastewater with similar properties (Jenicek et al. 2004; Svehla et al. 2014; Yang et al. 2010; Wei et al. 2014).

The economy of the process is strongly affected by the nitrogen oxidation rate (NOR) guaranteed by the system. Jenicek et al. (2004) observed that during long-term operation at 21 ± 1 °C, it is possible to maintain efficient partial nitritation of reject water in a SBR using flocculent biomass at a relatively high nitrogen loading rate of up to 1.65 kg/(m^3 day). Intensive fluctuation of FA and FNA concentration during SBR cycle was identified as the main factor restricting NOB activity in the experiments performed by Svehla et al. (2014) in a system with analogic technological arrangements operated at 23 ± 2 °C. Yang et al. (2010) achieved highly stable performance of partial nitritation applied to reject water treatment with the maximum NLR reaching even 4.2 kg/(m^3 day) at slightly higher temperatures (26 ± 4 °C). Subsequent studies proved that the SBR treating reject water under conditions comparable with Jenicek et al. (2004) and Svehla et al. (2014) is able to be sufficiently operated after a sudden fall in temperature from 24 to 17 °C, in the case that relatively low NLR reaching 0.2 kg/(m^3 day) is applied. Contrarily, the collapse of biological processes was observed after decreasing the temperature from 24 to 16 °C at the same NLR (Hrncirova et al. 2017). However, the function of the system after the intensive temperature falls at higher NLR was still not evaluated. Thus, the productivity of the system under fluctuating temperatures is still unknown.

The aim of the experiments presented within this paper is to evaluate the influence of NLR on the temperature resistance of the system treating reject water by partial nitritation, which is a potential precursor to denitrification via nitrite or ANAMMOX process. The research was performed under technological conditions analogical to our previous studies (Jenicek et al. 2004; Svehla et al. 2014; Hrncirova et al. 2017). The system was repeatedly exposed to a sudden temperature fall from 24 to 17 °C where NLR was increased incrementally from 0.4 to 1.5 kg/(m^3 day). The starting temperature (24 °C) was selected because it represented the upper limits of the temperature applied by Jenicek et al. (2004) and Svehla et al. (2014) during previous experiments. Temperature decreases to 17 °C were realized based on the findings presented by Hrncirova et al. (2017). The range of NLR tested was selected with the aim to evaluate the temperature stability of the system at NLR exceeding value 0.2 kg/(m^3 day) and approaching 1.65 kg/(m^3 day) achievable at a relatively constant temperature under comparable conditions (Jenicek et al. 2004). Thus, the ability of the system to be highly productive under temperature fluctuation was feasible to evaluate.

Theoretical

The concentrations of FA (C_{FA}) and FNA (C_{FNA}) were calculated in accordance with Park and Bae (2009) and Anthonisen et al. (1976):

$$C_{FA}(\mathrm{mg/L\ NH_3}) = \frac{17}{14}\frac{C_{TAN}\cdot 10^{pH}}{[\exp(6334/(273+T)+10^{pH})]} \quad (1)$$

$$C_{FNA}(\mathrm{mg/L\ HNO_3}) = \frac{47}{14}\frac{C_{TN^{III}N}}{[\exp(-2300/(273+T)+10^{pH})]+1}, \quad (2)$$

where C_{TAN} and C^{III}_{TNN} represent the actual total concentrations of TAN and $TN^{III}N$, respectively, and T is the temperature in degrees centigrade.

The actual value of SRT in days reached in the reactor was calculated in accordance with the Eq. (3):

$$\mathrm{SRT} = \frac{V\cdot \mathrm{TSS_R}}{Q\cdot \mathrm{TSS_{eff}}}, \quad (3)$$

where V is the volume of the reactor (0.75 L), TSS_R represents the concentration of TSS in g/L in the reactor, Q is the intensity of the feeding of the reactor (L/d) and TSS_{eff} describes TSS concentration (g/L) in the effluent.

Experimental

Reactor set-up

A poly methyl methacrylate laboratory model of a nitrifying reactor (0.75 L) with a flocculent biomass was operated in a thermostatic box ET 619-4 (Lovibond, Germany) for 330 days (Fig. 1). The experiment was realized in the period lasting from January to November 2015 at non-sterile conditions. The system was inoculated with an activated sludge gained from other laboratory model treating reject water using the partial nitritation operated in SBR under the conditions described by Svehla et al. (2014). The entire volume of the reactor was filled with this activated sludge. This way, the volatile suspended solids (VSS) concentration reached 2.4 g/L at the start of the reactor operation. The inoculum was gathered from the reactor treating reject water at a laboratory temperature (23 ± 2 °C) under the conditions comparable with the experiment presented in this paper. The only difference was that the reactor serving as the source of inoculum was not placed in a thermostatic box and the temperature fluctuated in the range mentioned above. Thus, the nitrifying organisms were satisfactorily adapted to the conditions prevailing in the reactor. It enabled starting the experiment immediately after the inoculation.

The SBR was operated in two 12-h cycles per day during periods 1–8. Taking into consideration the increased volume of reject water exchanged during one cycle resulting from increase of NLR, it was impossible to apply this strategy to SBR operation during periods 9 and 10. Therefore, four cycles per day were applied during these

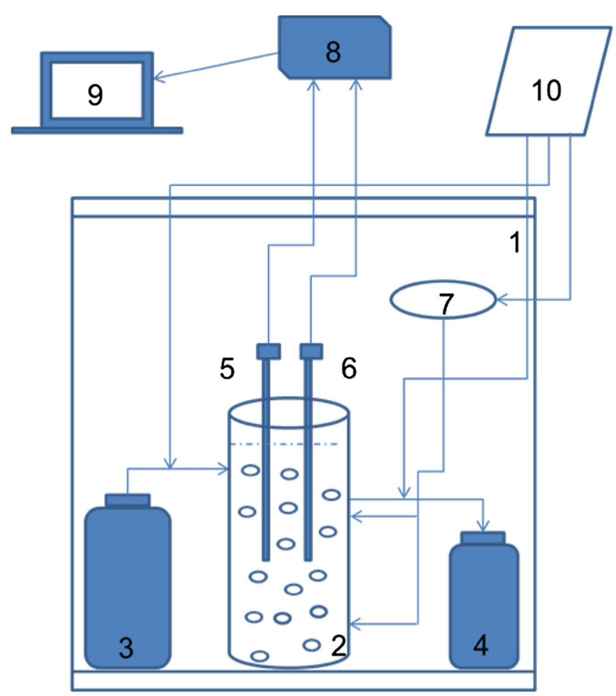


Fig. 1 Schematic diagram of SBR reactor—notes: *1* thermostatic box *2* SBR reactor, *3* reject water tank, *4* effluent water tank, *5* pH electrode, *6* DO electrode, *7* aeration, *8* interface, *9* PC, *10* electric time-switches

periods with each cycle lasting for 6 h. Each SBR cycle consisted of the following phases: inflow of treated water into the reactor (10 min); the working phase, when the reactor volume was aerated (11 h 20 min during periods 1–8; 5 h 20 min during periods 9 and 10); sedimentation (20 min); and drainage of effluent water (10 min). The duration of particular phases of SBR cycles were controlled using electric time-switches controlling the operation of peristaltic pumps and aerators.

In accordance with Jenicek et al. (2004) and Svehla et al. (2014), no excess sludge was withdrawn from the reactor, with the exception of sludge escaping with effluent water, with the aim to maintain the maximum of AOB in the reactor. The concentration of biomass expressed as total suspended solids (TSS) and VSS fluctuated in the range of 2.1 to 4.5 g/L and 1.8 to 3.0 g/L, respectively, during the whole reactor operation. The concentration of TSS in the effluent did not exceed 0.5 g/L. Under such conditions, the SRT calculated in accordance with the Eq. (3) fluctuated between 8 and 20 days.

Peristaltic pumps and silicone tubes were used to transport treated reject water into the reactor. The reactor was aerated using the coarse bubble system. A small aquarium air pump enabling the regulation of aeration intensity was used for this purpose. The volume of the reactor was intensively stirred by the supplying of the air into the reactor during the whole working phase of the reactor. The concentration of dissolved oxygen in the reactor was not limited with the aim to maximize NOR (Pacek et al. 2015). For this purpose the intensity of aeration was controlled with regard to actual NLR with the aim to achieve DO concentration exceeding 3 mg/L for the whole working phase of the cycle. As a consequence of gradual decrease of treatment process intensity during the cycle, certain increases of DO concentration were observed during the working phase of the cycle. In all cases, the concentration of DO reached an average of 3.3 and 7.8 mg/L at the beginning and at the end of working phase, respectively. Thanks to the aeration intensity control, DO concentration did not differ significantly in particular periods despite the changes of NLR applied.

Although the pH value was monitored continuously (see below), no neutralizing agents were added into the reactor. Under such conditions, pH fluctuated significantly during the SBR cycle as a consequence of the acidification of the environment during the partial nitritation process reaching maximum value at the beginning of the cycle working phase and the minimum value at the end of the working phase (Svehla et al. 2014). Thus, the system's pH was self-regulated in the process by the high alkalinity of the reject water.

The reactor was repeatedly exposed to temperature drops from 24 to 17 °C. Simultaneously, the NLR was increased incrementally from 0.4 to 1.5 kg/(m^3 day). The temperature fluctuation was simulated by changing the temperature on the thermostatic box from original to required levels. Under such conditions, required temperature was achieved after approximately 4 h in the reactor, at the change of particular operational periods. NLR reaching 0.4; 0.6; 1.0; 1.2 and 1.5 kg/(m^3 day) was applied during the experiment. NLR was regulated by the control of the raw reject water pumping intensity, where actual TAN concentration in raw reject water was taken into account. The operation of the laboratory model was divided into 10 periods according to the actual value of the NLR and temperature (Table 1). The starting temperature, the intensity of temperature decreases and the range of NLR applied were selected based on the results of the previous experiments (Jenicek et al. 2004; Svehla et al. 2014; Hrncirova et al. 2017). Duration of particular periods was selected with the aim to ensure sufficient time for the biomass acclimatization after temperature changes where the results of previous experiments (Hrncirova et al. 2017) were taken into consideration.

Table 1 Temperature, nitrogen loading rate and hydraulic retention time (HRT) at individual periods of reactor operation

Days	Operational period	Temperature [°C]	NLR [kg/(m^3 day)]	HRT [day]
0–16	1	24	0.4	2.8
17–33	2	17	0.4	2.8
34–68	3	24	0.6	2.1
69–109	4	17	0.6	2.1
110–122	5	24	1.0	1.2
123–164	6	17	1.0	1.2
165–213	7	24	1.2	1.0
214–248	8	17	1.2	1.0
249–289	9	24	1.5	0.8
290–330	10	17	1.5	0.8

Treated water

The reject water from the central wastewater treatment plant in Prague was used. Its quality is shown in Table 2.

Analytical methods

The spectrophotometric measurement of different nitrogen forms (TAN, $TN^{III}N$, $N–NO_3^-$) and chemical oxygen demand (COD) in raw reject water and in the effluent from the reactor was performed using a HACH (Hach Lange GmbH, Germany) DR/4000 photometer according to the standard methods (Eaton et al. 1995). The determination of total suspended solids (TSS) and VSS were measured gravimetrically according to standard methods (Eaton et al.

Table 2 Physico-chemical properties of treated reject water

Parameter	Unit	Average	Range
pH	–	8.4	8.1–8.7
Alkalinity	mmol/L	93	70–121
P total	mg/L	37	23–63
TAN	mg/L	1349	1055–1643
COD	mg/L	2700	1145–4600
COD soluble	mg/L	1532	770–2322
TSS	g/L	3.3	2.1–5.5
Molar ratio alkalinity/TAN	–	1.02	0.76–1.25

TAN total ammonium nitrogen, *COD* chemical oxygen demand, *TSS* total suspended solids

1995). P-total concentration was determined with a HACH (Hach Lange GmbH, Germany) DR/4000 photometer using HACH method number 8190. Alkalinity was determined by titration of the sample with hydrochloric acid (0.1 mol/L) up to pH 4.5. Temperature, pH and dissolved oxygen concentration were monitored continuously online using a Gryf Magic XBC device (Gryf HB company, Czech Republic). The value of pH was measured with an ISE electrode PCL 321 XB2 and DO was determinated with a membrane electrode KCL 24 XB4. Both types of electrodes were equipped with temperature sensors.

Fluorescence in situ hybridization

As per Daims et al. (2001), FISH was carried out to quantify the amount of AOB and NOB in the sludge from the reactor. The samples of activated sludge were fixed in a 4% paraformaldehyde solution for 3 h at 4 °C. Consequently, they were washed three times with phosphate-buffered saline (PBS) where centrifugation at 3500*g* for 8 min was applied with the aim to separate the flocs and supernatant (Nielsen et al. 2009). Microorganisms present in the flocs were hybridized by FISH according to Nielsen et al. (2009). AOB were identified using Nso190 and Nso1225 probes and NOB were identified using Ntspa712, Ntspa662 and NIT3 probes. The specificity of all FISH probes applied, probe sequences and fluorophores used for each probes are presented in Table 3.

FISH images were collected using an Olympus BX51-RFAA epifluorescence microscope with a charge-coupled device (CCD) camera. Photos were achieved in 2D at the surface of the floc.

Sludge was sampled on day 232 (period 8), day 253 (period 9) and day 325 (period 10). FISH was carried out at a specialized department (Department of Water Technology and Environmental Engineering, University of Chemistry and Technology Prague).

Results and discussion

Achievement of partial nitritation during experiment

$TN^{III}N$ represented 94 to 99% of oxidised nitrogen (the sum of $TN^{III}N$ and $N–NO_3^-$) in the effluent during the entire reactor operation (Table 4). $N–NO_3^-$ concentration did not exceed 45 mg/L (Fig. 2). Thus, successful partial nitritation was achieved. This finding is in accordance with Svehla et al. (2014), which proved that the fluctuation of FA and FNA concentration during SBR cycle is able to restrict NOB activity as the sole inhibiting factor where AOB activity could be preserved in nitrifying reactor treating reject water under the conditions applied in our experiment. Contrarily, in a case that a completely stirred tank reactor (CSTR) would be used, the inhibiting effect of FA and/or FNA would be necessary to combine with the other strategy limiting NOB activity, e.g. with controlled oxygen supply, with the aim to achieve partial nitritation (Pacek et al. 2015; Svehla et al. 2014).

Even temperature decreases from 24 to 17 °C realized at the beginning of periods 2, 4, 6, 8 and 10 did not result in a significant increase of $N–NO_3^-$ production, although faster growth of NOB at temperatures lower than 20 °C was reported by Hellinga et al. (1998) and Hao et al. (2002). The inhibition effect of FA and FNA (Svehla et al. 2014; Anthonisen et al. 1976; Vadivelu et al. 2007; Blackburne et al. 2008; Pambrun et al. 2008) seems to be sufficient to inhibit NOB, despite the temperature reached in the reactor favoured NOB in comparison with AOB (Hellinga et al. 1998; Hao et al. 2002). The washout of NOB during the experiment was proved by the results of Fluorescence In Situ Hybridization (see separate chapter focused on the results of microbial analysis).

$TN^{III}N$ and TAN concentrations reached 490 to 690 mg/L and 520 to 760 mg/L, respectively, throughout the whole reactor operation (Fig. 2). The average ratios of $TN^{III}N$ and TAN concentrations in the effluent from the reactor ranged between 0.90 and 1.02 within individual periods of the reactor operation where no significant changes of this parameter were registered after the temperature changes (Table 4). Observed $TN^{III}N$/TAN ratios are in agreement with other studies evaluating partial nitritation of reject water without pH control (van Dongen et al. 2001; Jenicek et al. 2004) being suitable for potential subsequent ANAMMOX processes.

Table 3 FISH analysis: specificity of all FISH probes, probe sequences, competitor oligonucleotides and fluorophores used for each probe

Probe name	Bacterial genera	Probe sequences	Competitor oligonucleotide	Fluorophores	References
Nso190	Betaproteobacterial ammonia-oxidising bacteria	CGA TCC CCT GCT TTT CTC C	None	Cy3, Fluos	Daims et al. 2006
Nso1225	Betaproteobacterial ammonia-oxidising bacteria	CGC CAT TGT ATT ACG TGT GA	None	Cy3, Fluos	Daims et al. 2006
Ntspa712	Phylum *Nitrospirae*	CGC CTT CGC CAC CGG CCT TCC	CGC CTT CGC CAC CGG TGT TCC	Cy3, Fluos	Mobarry et al. 1996
Ntspa662	Genus *Nitrospira*	GGA ATT CCG CGC TCC TCT	GGA ATT CCG CTC TCC TCT	Cy3, Fluos	Mobarry et al. 1996
NIT3	Genus *Nitrobacter*	CCT GTG CTC CAT GCT CCG	CCT GTG CTC CAG GCT CCG	Cy3, Fluos	Wagner et al. 1996

Table 4 % of $TN^{III}N$ within oxidised nitrogen and $TN^{III}N$/TAN ratio

Operational Period	$TN^{III}N/(TN^{III}N + N\text{-}NO_3^-)$ [%]	$TN^{III}N$/TAN
1	95.5 ± 0.6	1.00 ± 0.02
2	96.8 ± 0.2	1.01 ± 0.02
3	96.2 ± 0.3	1.00 ± 0.01
4	96.8 ± 0.7	1.00 ± 0.04
5	94.9 ± 0.6	1.01 ± 0.03
6	96.4 ± 0.6	0.90 ± 0.06
7	97.0 ± 0.6	0.92 ± 0.04
8	98.2 ± 0.3	1.02 ± 0.02
9	97.5 ± 0.6	0.91 ± 0.05
10	96.9 ± 0.5	0.90 ± 0.06

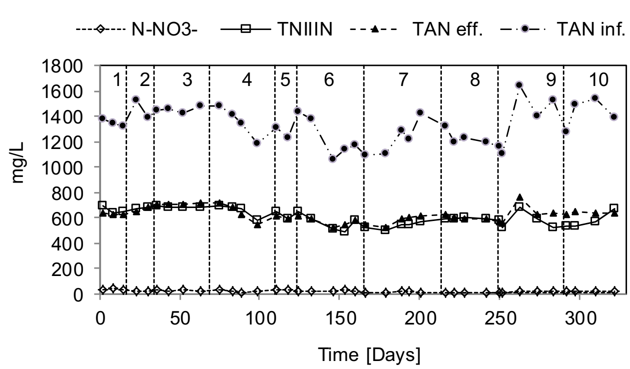


Fig. 2 Nitrogen species in the effluent (particular periods indicated using numbers 1–10)

Evaluation of partial nitritation stability according to nitrogen oxidation efficiency and nitrogen oxidation rate

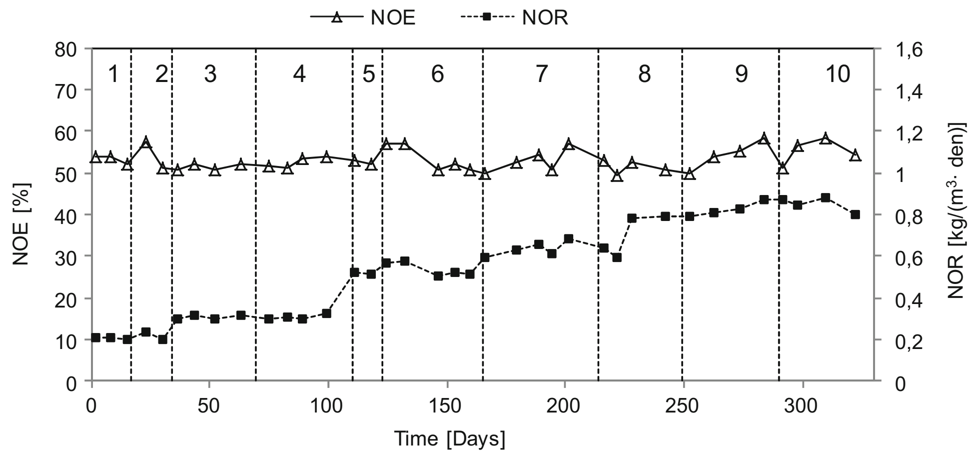
Nitrogen oxidation efficiency (NOE) reached 48 – 58% throughout the whole experiment (Fig. 3). This range of NOE is typical for successful partial nitritation of reject water without pH control (van Dongen et al. 2001; Jenicek et al. 2004). No substantial changes of NOE were registered after temperature falls at the turn of particular periods, indicating good stability of partial nitritation even after temperature decreases from 24 to 17 °C.

Taking into consideration relatively stable NOE during the experiment, the NOR increased proportionally to the NLR within particular periods of the reactor operation (Fig. 3). No apparent decrease in the NOR was registered after the temperature decreases at the beginning of periods 2, 4, 6, 8 and 10 indicating temperature stability of the system within the tested NLR range. During the first phase of the experiment (periods 1 and 2), the NOR reached 0.20 kg/(m^3 day) on average. The maximum NOR reaching 0.84 kg/(m^3 day) on average was achieved during periods 9 and 10. SHARON process as one of the most commonly applied systems for reject water treatment in a full scale at present time (Lackner et al. 2014) is usually operated at NLR lower than 1 kg/(m^3 day) (van Kempen et al. 2001). For subsequent ANAMMOX process ca. 50–60% of TAN should be oxidised to $TN^{III}N$ (van Dongen et al. 2001). The results presented above indicate that our reactor is able to achieve slightly higher NOR comparing with the SHARON reactor integrated into the system SHARON/ANAMMOX, despite the fact that significantly lower temperatures are applied. Additionally, strong fluctuation of temperature did not result in noticeable decreases of NOR.

Evaluation of partial nitritation stability according to pH profile during SBR cycle

H^+ is produced during nitrification process in the phase of nitrite production (Henze et al. 2008). In the case of the nitrification (and partial nitritation as well) of wastewater loaded with high amounts of nitrogen, such as reject water, the pH decreases during the treatment

Fig. 3 NOE and NOR at individual periods of reactor operation (particular periods indicated using numbers 1–10)



process. Insufficient alkalinity for such intensive H^+ production causes this phenomenon (Jenicek et al. 2004; Henze et al. 2008). As a consequence, significant pH fluctuations can be registered during the operational cycle of our SBR system when stable partial nitritation took place within this system (Svehla et al. 2014). Hrncirova et al. (2017) registered pH value in the range of 6.9–7.9 at the beginning of the SBR cycle working phase during the reject water treatment where the decrease to 4.7–6.5 was monitored at the end of working phase when partial nitritation was satisfactory operated. Consequently, the collapse of the system due to overly radical decrease of temperature was indicated by a pH increase to 8.9 where no pH fluctuation during SBR cycle was observed. Secondly, as a consequence of this pH increase, AOB were greatly inhibited by an extreme increase of FA concentrations reaching up to 150 mg/L.

In the experiment described in this paper, the pH value ranged between 7.6 and 8.2 at the beginning of working phase of the SBR cycle during the entire operation of the reactor (phases 1–10). During the SBR cycle the decrease of pH was observed as 5.2–6.2 measured at the end of the working phase (Fig. 4a). No significant changes in the pH profile were registered during the SBR cycle after sudden temperature decreases from 24 to 17 °C, realized throughout the experiment at different NLRs (at the turn of periods 1 and 2, 3 and 4 (Fig. 4b), 5 and 6, 7 and 8, 9 and 10 (Fig. 4c). Only a slight increase in pH (several tenths of a unit at maximum) was registered as an immediate reaction of the system to the temperature falls (Fig. 4b, c). Nevertheless, the strong fluctuation of pH levels was preserved even at a NLR reaching 1.5 kg/(m^3 day) (Fig. 4c). This finding indicates intensive AOB activity and a stable partial nitritation regardless of the changes of NLR and temperature under the conditions applied in this experiment.

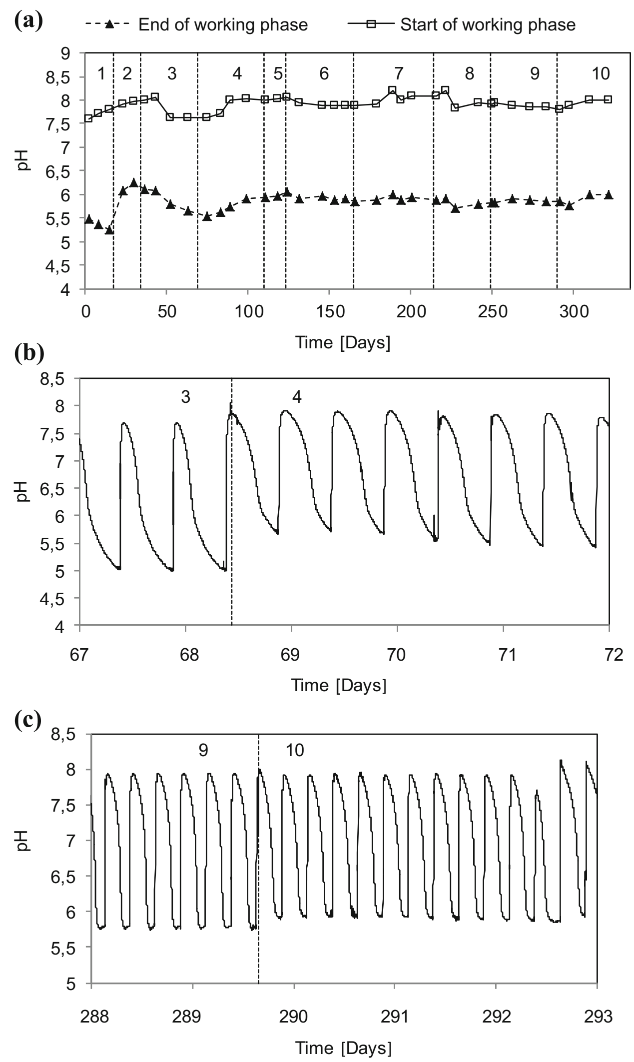


Fig. 4 pH value (particular periods indicated using numbers 1–10). **a** Measured weekly during whole reactor operation. **b** Measured continually at the turn of periods 3 and 4. **c** Measured continually at the turn of periods 9 and 10

Inhibiting pressure of FA and FNA towards AOB and NOB

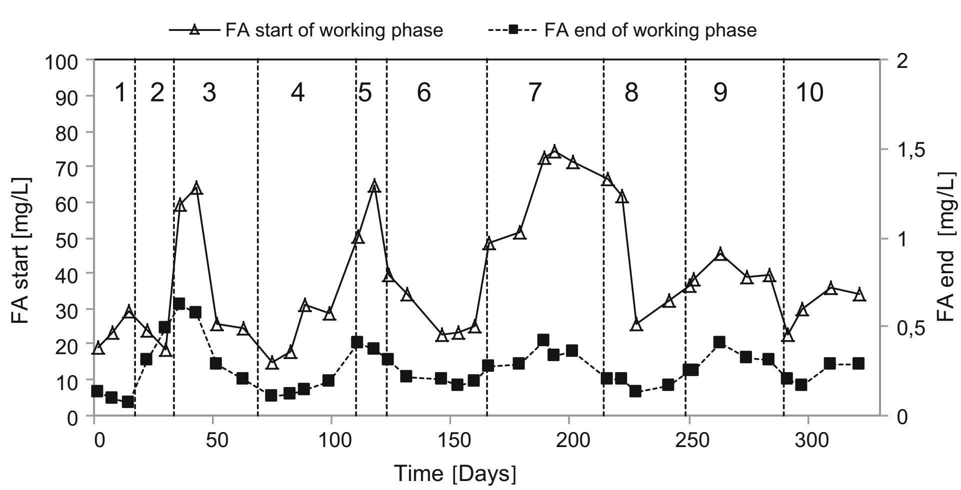
FA concentration reached maximum levels at the beginning of the working phase of SBR cycle (15 to 74 mg/L, Fig. 5), while FNA concentration increased during the cycle, reaching maximum levels at the end of the working phase (3.5–22 mg/L, Fig. 6). Under such conditions, the inhibition limits of FA (0.1–1.0 mg/L) and FNA (0.2–2.8 mg/L) for non-adapted *Nitrobacter* representing NOB (Anthonisen et al. 1976; Vadivelu et al. 2007) were significantly exceeded. Simultaneously, observed values were significantly higher than the values that enable NOB inhibition, even in long-term basis operated reactors for partial nitritation. In the experiment described by Svehla et al. (2015), FA concentration reaching 7 mg/L at the beginning of the SBR cycle combined with an FNA concentration reaching ca. 1 mg/L at the end of the cycle was proved to be sufficient for effective inhibition of NOB during partial nitritation of diluted reject water in SBR. As a result of the inhibition pressure caused by FA and FNA, the NOB activity was reliably inhibited within the experiment described in this paper.

On the contrary, AOB remained active despite the fact that the inhibition limit for FA reaching 10–150 mg/L (Anthonisen et al. 1976; Mosqura-Corral et al. 2005) was approached (Fig. 5). Even exceeding the FNA inhibition limits 0.1–0.63 mg/L N-HNO_2 (Vadivelu et al. 2006) corresponding with 0.3–2.1 mg/L FNA (Fig. 6) did not result in the destruction of partial nitritation. Satisfactory operation of partial nitritation presented in this study seems to be enabled by the ability of AOB to adapt to FA and FNA concentrations significantly higher than the inhibition limits effective for non-adapted cultures (Turk and Mavinic 1989; Villaverde et al. 2000; Zhou et al. 2011). Simultaneously, some observations indicate that different species and strains within a genus of AOB tolerate different levels of FNA (Zhou et al. 2011). Therefore, certain microbial population shifts leading to a highly tolerant culture cannot be excluded. The ability of AOB to be active at FA and/or FNA concentration strongly exceeding inhibition limits for non-adapted cultures is in accordance with the findings of different authors evaluating partial nitritation process in wastewater loaded with high amounts of nitrogen. Maximum FA and FNA concentrations reached within our experiment are comparable with Svehla et al. (2014) who operated partial nitritation of reject water at the concentrations as high as 38 and 7 mg/L for FA and FNA, respectively. Also Sun et al. (2013) reported satisfactory activity of AOB at FA and FNA concentration exceeding 50 and 2 mg/L, respectively, in a system applying nitritation/denitritation for urban landfill leachate treatment. Wei et al. (2014) achieved satisfactory partial nitritation at FA concentration reaching 86.3 ± 3.29 mg/L. Hrncirova et al. (2017) observed no fatal effect of FNA concentration, even exceeding 60 mg/L within the operation of SBR treating reject water on the partial nitritation principle.

NOE was limited to approximately 50% during the entire reactor operation. This finding indicates that AOB were in some way inhibited at a certain FNA concentration at the end of working phase of SBR cycle in the environment with pH gradually decreasing and FNA concentration gradually increasing during the cycle. Consequently, they were probably reactivated at the beginning of subsequent cycle in connection with the pH increase and simultaneous FNA concentration decrease. This hypothesis is in accordance with Claros et al. (2013) which evaluated the effect of pH and FNA on the activity of AOB in the reactor applying partial nitritation.

Actual concentration of FA is determined by TAN concentration, pH and temperature (Eq. 1). Similarly, FNA concentration is influenced by $TN^{III}N$ concentration, pH and temperature according to Eq. 2. (Park and Bae 2009; Anthonisen et al. 1976). The highest FA concentrations

Fig. 5 FA concentrations at the start and at the end of working phase (particular periods indicated using numbers 1–10)



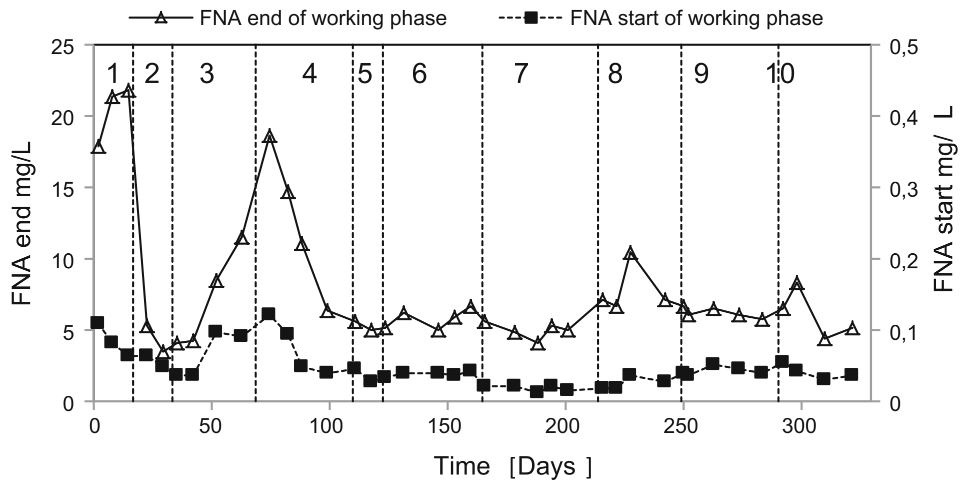


Fig. 6 FNA concentration at the start and at the end of working phase (particular periods indicated using numbers 1–10)

were observed during periods 3, 5, 7 and 8 where the values up to 64, 65, 75 and 66 mg/L, respectively, were reached Fig. 5). Contrarily, the highest FNA concentrations were achieved during periods 1, 3 and 4 reaching up to 22, 11 and 19 mg/L, respectively (Fig. 6). The pH value seems to be the most important factor determining maximum FA and FNA concentration during SBR cycle (Fig. 4). In contrast, the effect of temperature fluctuation simulated within particular periods seems to be relatively insignificant.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (Fig. 7) showed that AOB prevail over NOB. AOB accounted for 27.2% of the total biomass in period 8, 13.6% in period 9 and 32.1% in period 10. Variable concentrations of organic compounds expressed by COD in treated reject water (see Table 2) probably resulted in variable representation of organotrophic bacteria in sludge and consequently to variable concentrations of total biomass. In agreement with this assumption, TSS concentrations fluctuated significantly during the experiment (2.1–4.5 g/L). This phenomenon seems to be responsible for the observed fluctuation of the representation of AOB in activated sludge.

NOB accounted for 2.0% in period 8; in period 9 and 10 the representation of NOB was lower than the detection limit (0.1%). Very low representation of NOB in activated sludge is in accordance with the accumulation of nitrites observed in the reactor. On the other hand, presence of N-NO_3^- in the effluent from the reactor (even though in very low concentration, Fig. 2) indicates certain activity of NOB in accordance with FISH result of period 8. The occurrence of NOB in our reactor is in accordance with Ganigué et al. (2009) which demonstrated presence of *Nitrobacter* and *Nitrospira* even in the reactor treating leachate with extremely high TAN and $TN^{III}N$

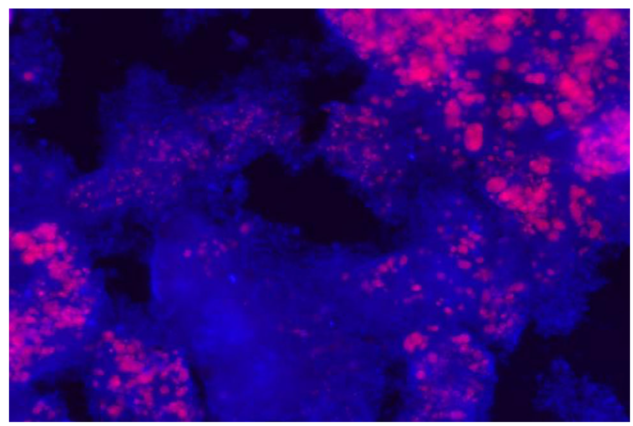


Fig. 7 FISH image of the biomass: pink colour signifies AOB targeting by probes Nso190, Nso1225, NOB were not detected. Enlargement: 320 x, sample taken on day 325 (period 10)

concentrations despite the fact that effective nitrite accumulation was achieved.

Potential implications of results for operation of full-scale reactors applying partial nitritation

Jenicek et al. (2004) proved that SBR treating reject water under conditions analogical to this study could be successfully operated at a NLR of up to 1.65 kg/(m^3 day) in the case that the temperature 21 ± 1 °C is applied. Following experiment performed by Hrncirova et al. (2017) proved that the system is able to tolerate sudden temperature falls from 24 to 17 °C, whereas the temperature decreases from 24 to 16 °C led to significant disruption of the treatment process stability at low NLR reaching 0.2 kg/(m^3 d). New results presenting within this paper indicate that the system is able to tolerate sudden temperature falls from 24 to 17 °C even when the NLR is approaching maximum values achieved by Jenicek et al. (2004). Thus, the possibility of operating a robust system for the partial nitritation of reject water or other types of wastewater with extremely high TAN concentration under widely fluctuating temperatures was confirmed. In addition, partial nitritation was operated satisfactorily at NLR 1.5 kg/(m^3 d) for 40 day at a constant temperature of 17 °C within operational period 10. This indicates the possibility of operating the tested system at simultaneously low temperatures and high NLR on a long-term basis. Considering the fact that also the possibility of the ANAMMOX process operation at fluctuating temperature significantly lower than the optimum treatment value was proved within some studies (Zekker et al. 2014, 2016), it seems to be possible to operate satisfactory even the system applying partial nitritation/ANAMMOX under unstable temperature conditions.

Combining older results with the findings presented in this paper, it is possible to conclude that the intensity of sudden temperature falls (or more precisely the final value of temperatures reached after the fall from a strictly monitored initial temperature value) influences the stability of the treatment process much more significantly than the NLR. The temperature in very narrow range of 16–17 °C seems to be critical from this point of view under applied conditions.

Maximum NLR applied successfully in this study exceeds values reached in many current full-scale systems for reject water treatment. For example the SHARON process is usually operated at a NLR below 1 kg/(m^3 day) and simultaneously at temperature reaching 30–40 °C (van Kempen et al. 2001; Lackner et al. 2014) which significantly exceed temperatures applied in this study. Thus, the system presented within this paper represents a promising variant for the treatment of wastewaters loaded with high amounts of nitrogen. NLRs that were significantly higher than the values achieved within this study were successfully applied by some authors in a laboratory scale. For example, Yang et al. (2010) achieved highly stable performance of partial nitritation with a maximum loading rate reaching 4.2 kg/(m^3 day). Even NLR 5.0 ± 1.0 kg/(m^3 day) enabled the stable operation of partial nitritation within the experiment described by Torà et al. (2014). Considerably higher operational temperatures (26 and 30 °C, respectively) were applied by Torà et al. (2014) and Yang et al. (2010). In addition, the effect of potential temperature fluctuations was not evaluated within the cited studies.

It is impossible to perfectly simulate the natural ambient temperature fluctuations at a laboratory scale. Sudden decreases in temperature from 24 to 17 °C were applied within this experiment, where the starting temperature was transformed into the required level over approximately 4 h. Such quick changes of temperature seem not be realistic in practice. Taking into consideration common fluctuations of ambient temperature and the high temperature typical for raw reject water, it is reasonable to expect less intensive fluctuations of temperature in a full-scale reactor. Under such conditions it can be assumed that the system will be able to tolerate even lower temperatures. In accordance with this assumption, Hrncirova et al. (2017) proved that it is possible to operate partial nitritation of reject water satisfactorily even after gradual temperature falls from 24 to 14.5 °C, in the case that the temperature was decreased incrementally for 12 days at NLR reaching 0.2 kg/(m^3 day). Similarly, Persson et al. (2014) confirmed the possibility of successfully applying the partial nitritation/ANAMMOX process to reject water treatment in MBBR after gradual temperature decreases from 19to 13 °C.

Conclusions

A high-performance system for reject water treatment able to tolerate strong fluctuations of temperature was presented within this study. The stability of partial nitritation operated in a SBR with biomass cultivated in the form of activated sludge was not disrupted even after sudden temperature decrease from 24 to 17 °C realized at a NLR reaching 1.5 kg/(m^3 day). Simultaneously, it was found that $TN^{III}N$ was a dominant oxidised nitrogen form regardless of the temperature fluctuation. A strong inhibition effect of FA and FNA was responsible for the restriction of NOB activity under the conditions applied. The authors plan to realize subsequent pilot plant experiments with the aim to simulate better real conditions and to verify the results gained in the laboratory experiments. Simultaneously, the temperature resistance of the systems nitritation/denitritation and partial nitritation/ANAMMOX is planned for the future.

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