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Rapid achievement of nitrification in CSTR and SBR treating reject water at high ammonia levels

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ABSTRACT

The ability of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) to carry out nitrification in an environment with high ammonia levels was evaluated with the aim to suggest a suitable strategy for a quick start-up of nitrifying reactors treating reject water. It was proved that nitrification could be initiated even without any inoculation of nitrifying biomass. The nitritation was achieved faster (23 d after beginning of reactor operation) than nitratation (after 180 d) due to the presence of AOB and the absence of NOB in raw reject water. Free ammonia (FA) concentration reaching up to 160 mg N L^{-1} strongly inhibited nitrifying bacteria during the initial phase of reactor operation. Consequently, free nitrous acid (FNA) with a concentration up to 1.25 mg N L^{-1} supported NOB inhibition after the initiation of AOB activity. High AOB, as well as NOB, activity was observed even during the first day of the operation in a completely stirred tank reactor (CSTR, nitrogen loading rate—NLR 0.5 kg N $(m^3\ d)^{-1}$, nitrogen oxidation rate—NOR 0.25 ± 0.05 kg N $(m^3\ d)^{-1}$) as well as a sequencing batch reactor (SBR, NLR 0.3 kg N $(m^3\ d)^{-1}$, NOR 0.14 ± 0.01 kg N $(m^3\ d)^{-1}$) inoculated with common activated sludge at a zero initial total ammonium nitrogen concentration. It was proved that temporary inhibition of NOB activity induced by FA concentration elevated to 88 mg N L^{-1} may lead to the permanent transformation of standard nitrification into shortcut nitrification in SBRs, thanks to periodical exposition of NOB to high FA and FNA concentration during SBR cycle. Thus, it was demonstrated that the possibility to initiate this process very quickly is essential as a first stage for subsequent anaerobic oxidations of ammonium or shortcut denitrification.

Keywords: Shortcut nitrification; Start-up; Free ammonia; Free nitrous acid; Sequencing batch reactor (SBR); Completely stirred tank reactor (CSTR); Inhibition; Substrate spiking

1. Introduction

Biological nitrogen removal technologies applied in wastewater treatment are usually based on conventional nitrification/denitrification processes. This method is also applicable for separate treatment of reject water produced during the thickening and dewatering of anaerobically digested sludge [1]. However, the specific composition of this type of wastewater (extremely high total ammonium nitrogen—TAN

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concentration, low chemical oxygen demand/nitrogen —COD/N ratio, and higher pH) makes it very suitable for alternative processes, such as shortcut nitrification followed by denitrification or by anaerobic oxidation of ammonium (ANAMMOX) [2–6]. Shortcut nitrification consists of nitrite accumulation and is based on the inhibition of nitrite-oxidizing bacteria (NOB), while the activity and growth of ammonia-oxidizing bacteria (AOB) remains preserved. This is achieved because of the higher sensitivity of NOB toward particular physical–chemical factors, such as low dissolved oxygen (DO) concentrations [7,8], the combination of a short sludge retention time (SRT), and high temperature [9] or toxic nitrogen forms, such as free ammonia (FA) and free nitrous acid (FNA) [10,11]. The inhibiting concentration of FA ranges, depending on different published articles, within 0.1–3 mg N L^{-1} for NOB and within 8.2–124 mg N L^{-1} for AOB [11–13], respectively. The inhibiting concentrations for FNA are significantly lower. NOB were inhibited within the range of 0.02–1.35 mg N L^{-1}, while AOB seems to be slightly more tolerant to inhibiting values ranging from 0.30 to 2.13 mg N L^{-1} [11,14,15]. The inhibition effect on NOB caused by FA and/or FNA is strongly affected by an applied feeding strategy in the operation of nitrifying reactors treating reject water [16]. The selective inhibition of NOB induced by strong fluctuations of FA and FNA concentration in a nitrifying reactor treating reject water is one relatively simple strategy leading to shortcut nitrification in a sequencing batch reactor (SBR) operation [11,16]. The researchers [17] proved that a reactor applying shortcut nitrification for separate reject water treatment and operated with suspended biomass (activated sludge) on a SBR principle is able to remove TAN efficiently at NLR up to 1.65 kg N $(m^3\ d)^{-1}$ at 21 ± 1°C, reaching nitrogen oxidation rate (NOR) exceeding 1.5 kg N $(m^3\ d)^{-1}$ without any limitation of SRT. Using pH control nitrogen oxidation efficiency (NOE) exceeding even 90% was achieved within this experiment [17]. In the case of a continuous stirred tank reactor (CSTR), the effect of FA and/or FNA has to be combined with other factors in order to suppress NOB activity on a long-term basis [16]. Limited NOE (most frequently ca. 50–60%) is typical for reject water treatment by nitrification or shortcut nitrification [16,17]. Insufficient alkalinity causing decrease in pH is responsible for this phenomenon [18]. As a result, AOB activity is stopped just at the moment when approximately 1/2 of TAN is oxidized and pH decreases to approximately 6 [16,17].

Even though full nitrification or shortcut nitrification of reject water, once established, is a relatively robust treatment technology, the start-up process is often problematic. Raw reject water is characterized by pH values at around 8 and TAN concentrations reaching hundreds or thousands of mg L^{-1} [1]. An additional increase in pH up to 9 has to be expected due to CO_2 stripping during the intensive aeration of the activated mixture [19]. Under such conditions, the biomass is exposed to extremely high FA concentrations of tens or even hundreds of mg N L^{-1} [12]. Such concentrations not only cause the inhibition of more sensitive NOB (which would be favorable in order to achieve shortcut nitrification), but could also lead to the inhibition of AOB [11,12]. Naturally, the full or shortcut nitrification start-up phase duration is significantly influenced by the abundance of nitrifying bacteria in the inoculum, which in the case of common activated sludge ranges between 3 and 10% of total biomass [20], and by the actual NLR with respect to potential overloading of the system. Certain microbial population shifts leading to gradual gaining of a highly tolerant culture could be expected during the start-up phase of the operation of nitrifying reactors treating highly nitrogen-loaded wastewater treatment under specific conditions. For example, some observations indicate that different species and strains within a genus of AOB tolerate different levels of FNA [21]. Many results from experimental or full-scale systems (regardless of whether they are full nitrification/denitrification, shortcut nitrification/denitrification, or shortcut nitrification combined with ANAMMOX systems) treating ammonium-rich wastewaters cause the reactor start-up process to be time-consuming (3–4 months) [22–25]. In order to accelerate the start-up process, various strategies have been reported. Jubany et al. [26] applied the strategy consisting of an automated control of oxygen uptake rate, which could shorten the full nitrification process initiation period from 100 to 30–40 d. Another option described was bioaugmentation of biological reactors treating TAN-concentrated wastewaters [27,28]. With this method, Bartrolí et al. [27] shortened the initial period from 100 to 25 d, while gradually increasing NLR from 0.1 to 0.7 kg N $(m^3\ d)^{-1}$. The start-up process of nitrifying reactors treating reject water or other types of wastewater with extremely high TAN concentrations has been described repeatedly in the past. However, a comprehensive study evaluating the effect of different factors on the duration of the start-up phase and distribution of the final products of nitrification is still missing.

The objective of the experiment presented in this paper was to evaluate and compare different strategies for the start-up process of nitrifying reactors treating reject water. The aim of this study was to suggest an optimal strategy regarding the acceleration of the

initial reactor operation phase taking into account an extremely high TAN concentration in raw reject water. Moreover, the possibility of the start-up process acceleration of shortcut nitrification as a pretreatment for subsequent ANAMMOX or shortcut denitrification was assessed. Firstly, the start-up realized without any inoculation was monitored. Consequently, the influence of the method of inoculation, applied NLR, initial TAN concentration in the reactor, and the feeding strategy (CSTR and SBR) on the AOB and NOB activities were evaluated.

2. Materials and methods

2.1. Experimental setups

The model reactors (1.5 L) were designed as either a CSTR or as a SBR, where the technological schemes of the reactors were analogical to Svehla et al. [16]. A settler (0.25 L) ensuring sludge sedimentation was attached to the CSTR. Separated sludge was recirculated from settler back to the CSTR system using peristaltic pump. The SBR was operated in four 6-hour cycles per day. 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 (5 h 20 min); sedimentation (20 min); and drainage of efluent water (10 min). Duration of particular SBR phases was controlled using electric time switches regulating the operation of peristaltic pumps and aerator. All experiments were performed at room temperatures (23 ± 2˚C). All experiments were performed at room temperatures (23 ± 2˚C). Nitrifying activated sludge from the central wastewater treatment plant (WWTP) in Prague, Czech Republic, 10,00,000 population equivalent, was used as an inoculum in all experimental reactors excluding CSTR 1 (Table 1). No inoculum was used for the start-up of CSTR 1. The reactor was filled only with diluted reject water. Reject water itself served as the source of nitrifying biomass during the initial phase. Activated sludge from all operated model reactors showed good separation properties. Sludge volume index does not exceed 100 mL g^{-1} within all performed experiments. Raw reject water originated from the dewatering of sludge anaerobically digested under thermophilic conditions at the central WWTP in Prague was treated in the model reactors. Peristaltic pumps were used for transport of reject water into model reactors. Aeration was provided by air pump Sera air 550 R plus with adjustable intensity of aeration. Based on older findings indicating that potential DO limitation could induce the decrease in NOE under given conditions [8], DO concentration was maintained at values exceeding 3 mg L^{-1}. Depending on actual conditions (aeration intensity, NLR, sludge concentration, etc.), it ranged between 3.5 and 7.5 mg L^{-1} in all reactors. No excess sludge was withdrawn from the reactors in accordance with Jenicek et al. [17] and Svehla et al. [16]. In accordance with [16], the pH value was not artificially adjusted by a dosing of alkali. Under such conditions, NOE was limited by insufficient alkalinity of the treated reject water [17]. All experiments were performed using biomass cultivated in the form of suspension (activated sludge). Four start-up strategies were applied during the experiment. The start-up strategies differed mainly in the method of inoculation and in the initial TAN concentration. Three different variants were simulated in CSTR system from these perspectives. Taking into consideration the different inhibition pressures of FA and FNA depending on the feeding strategy [16], the start-up processes of reactors

Table 1
Initial setup of the experimental reactors

Model no.	Medium (Inoculum)	Int. TAN [mg L^{-1}]	Int. VSS [g L^{-1}]	Int. NLR [kg N $(m^3\ d)^{-1}$]	sNOR[a] [mg N (g VSS $d)^{-1}$]	Av. NLR [kg N $(m^3\ d)^{-1}$]	SRT [d]
CSTR 1	RW + TW (1:1)	560	0.1	0.2	440 ± 60	0.2 ± 0.05	12–20
CSTR 2	RW + AS (1:1)	630	2.7	0.2	130 ± 20	0.2 ± 0.03	8–25
CSTR 3	AS	<1	5.5	0.5	240 ± 30	0.5 ± 0.05	6–12
SBR	AS	<1	5.2	0.3/0.5	220 ± 30/370/40	0.3 ± 0.02/0.5 ± 0.04	5–14

Notes: AS—activated sludge; RW—reject water TW—tap water; NLR—Nitrogen loading rate; VSS—Volatile suspended solids; sNOR—Specific nitrogen oxidation rate; SRT—Sludge retention time.

[a]The highest concentrations of VSS were measured at the moment of initiation of inoculated reactors operation (CSTR 2, CSTR 3, and SBR) due to the addition of concentrated inoculum. The concentrations then decreased gradually as the consequence of heterothrophic biomass decay. The sNOR and SRT values were calculated from the values obtained after 14 d initial period.

operated in CSTR and SBR modes were also compared. The overview of the reactors simulating different start-up strategies is presented in Table 1.

2.2. Reject water composition

Reject water was obtained from a central WWTP plant in Prague. The physicochemical characteristics are shown in Table 2.

2.3. Analytical methods

The pH value, DO concentration, and temperature were monitored online (Gryf Magic XBC device, Gryf HB company, Czech Republic); pH was measured by ion selective electrode PCL 321 XB2 and DO was measured by a membrane electrode KCL 12 XB4 (Gryf HB company, Czech Republic). Both types of electrodes were equipped with temperature sensors. The concentration of nitrogen compounds, COD_{TS}, and COD_{DS} was measured weekly using HACH DR/4000 spectrophotometer (HACH LANGE GmbH, Germany). The analysis of TAN, N-NO_2^-, and COD_{TS} and COD_{DS} was performed in accordance with standard methods [24], N-NO_3^-, and P-tot concentrations were analyzed according to the HACH 2511 and 8190 methods, respectively (HACH-LANGE GmbH, Germany). COD_{TS} was determined in a homogenized sample, while in order to determine COD_{DS}, the sample was centrifuged at 9,500 rpm for 12 min before the analysis. The samples were taken from the influent and effluent reject waters. Analyses of biomass concentration (VSS) were performed according to the standard methods [29]. The alkalinity was determined by titration with HCl (0.1 mol L^{-1}) up to pH 4.5 according to the standard methods [29].

2.4. *Fluorescence* in situ *hybridization*

In order to reveal the microbial composition of the nitrifying biomass, the fluorescence *in situ* hybridization (FISH) analysis was performed. AOB communities were examined by the NSO_mix (NSO190 + NSO1225) probe detecting (β-proteobacterial ammonium-oxidizing bacteria) and identified by stain Fluos, green color. NOB was quantified by the Ntspa_mix (Ntspa712 + Ntspa662) probe to detect the whole phylum *Nitrospirae* species and NIT3-detecting *Nitrobacter* species, stain Cy3, and red color. The same stain, however, was used for the quantification of AOB biomass in the sample of raw reject water. FISH images were collected using an Olympus BX51-RFAA epifluorescence microscope with a charge-coupled device camera. FISH was performed according to Daims et al. [30].

2.5. Calculations

The concentrations of FA (C_{FA}) and FNA (C_{FNA}) were calculated in accordance with Anthonisen et al. [12] and Park and Bae [11]:

$$C_{FA}(\text{mg N L}^{-1}) = \frac{C_{TAN}\,10^{pH}}{[\exp(6334/(273+T)) + 10^{pH}]} \quad (1)$$

$$C_{FNA}(\text{mg N L}^{-1}) = \frac{C_{N\text{-}NO_2^-}}{[\exp(-2300/(273+T)) + 10^{pH}] + 1} \quad (2)$$

where C_{TAN} and $C_{N\text{-}NO_2^-}$ represent the actual total concentrations of TAN and N-NO_2^-, respectively, and T is the temperature in degree centigrade.

NOE was calculated according to Eq. (3):

$$\text{NOE}\ (\%) = \frac{C_{N\text{-}NO_2^-} + C_{N\text{-}NO_3^-}}{C_{TN-effl.}} \quad (3)$$

where $C_{N\text{-}NO_2^-}$ and $C_{N\text{-}NO_3^-}$ symbolize concentration of N-NO_2^- and N-NO_3^-, respectively measured in the effluent and $C_{TN\text{-}effl.}$ is the concentration of total nitrogen forms present in the effluent.

NOR, kg N $(m^3\ d)^{-1}$ was calculated from NLR [kg N $(m^3\ d^{-1})$ and NOE according to:

$$\text{NOR} = \frac{\text{NLR} \times \text{NOE}}{100} \quad (4)$$

Table 2
Parameters of the influent reject water

pH	TAN (mg N L^{-1})	P_{tot} (mg L^{-1})	Alkalinity (mmol L^{-1})	COD_{TS} (mg L^{-1})	COD_{DS} (mg L^{-1})	TSS (g L^{-1})
8.42 ± 0.22	1,190 ± 185	34 ± 8	97 ± 6	2,685 ± 770	1,665 ± 510	1.14 ± 0.05

Notes: P_{tot}—total phosphorus; COD_{TS}—chemical oxygen demand of total solids; COD_{DS}—chemical oxygen demand of dissolved solids; TSS—total suspended solids.

2.6. Dates and location of the research

The experiment was performed at Czech University of Life Sciences Prague, Department of Agro-Environmental Chemistry and Plant Nutrition from 17th September, 2013 to 28th July 2014. The FISH analyses were conducted at the University of Chemistry and Technology Prague, Department of Water Technology and Environmental Engineering.

3. Results and discussion

3.1. Start-up process in CSTR without inoculum

CSTR 1 without inoculum was filled only with diluted reject water. The average VSS during the experiment was extremely low (0.14 ± 0.10 g L^{-1}). A very low nitrogen (as well as organic) loading rate seems to be responsible for this observation. The initial pH of 8.9 was caused by the aeration in the reactor, which stripped out CO_2 accumulated during anaerobic digestion [19]. At an initial TAN concentration reaching 560 mg L^{-1} (Fig. 1(A)), it resulted in FA concentration of up to 160 mg N L^{-1} (Fig. 1(C)), significantly exceeding the inhibition limits for NOB and also for AOB [11,12]. Nevertheless, a decrease in pH to 6.7 on day 23 (Fig. 1(A)) indicates intensification of AOB activity in this phase of the reactor operation [17,18]. As a consequence of AOB activity, the increase in $N\text{-}NO_2^-$ to 346 mg L^{-1} was detected on day 23. Thus, the nitrifying biomass (especially NOB) was secondarily inhibited also by FNA [12,21]. Also potential nitrite inhibition toward denitrification and ANAMMOX bacteria must be taken into account in the case of subsequent treatment of the effluent in the denitrification or ANAMMOX reactor [31,32]. Further development in the reactor was characterized by a stable accumulation of $N\text{-}NO_2^-$. Since the day 50 onwards, a gradual increase in $N\text{-}NO_3^-$ concentration was observed indicating slow establishment of NOB activity. It resulted in vanishing of $N\text{-}NO_2^-$ in the effluent observed at day 191 (Fig. 1(A)). After the termination of the initial period of reactor operation (since day 23 onwards), the level of NOE reached $53.9 \pm 4.2\%$, which resulted, at a given NLR of 0.20 ± 0.05 kg N $(m^3\ d)^{-1}$, in NOR of 0.11 ± 0.03 kg N $(m^3\ d)^{-1}$ (Fig. 1(B)). The NOE and, thus, also the NOR is generally limited by an insufficient ratio between alkalinity and TAN concentration in treated reject water [17].

FA concentration decreased from 160 to 0.33 mg N L^{-1} during first 23 d of the reactor operation (Fig. 1(C)). Simultaneously, FNA concentration increased gradually reaching 0.55 ± 0.30 mg N L^{-1} between the days 23 and 181 due to increased $N\text{-}NO_2^-$ production and decrease in pH (see above). Gradual recovery of AOB and NOB activities, even at concentration exceeding inhibition limits of FA and FNA, were enabled by the adaptation of nitrifying bacteria to conditions prevailing in the reactor [21,31].

The results clearly demonstrate that the start-up of shortcut nitrification is feasible without purposed biomass inoculation, which indicates the presence of AOB in the raw reject water. This observation was supported by the FISH analysis performed (Section 3.7).

3.2. Start-up process in CSTR with inoculum and high initial TAN concentration

Another option for a reactor start-up was demonstrated via CSTR 2. This system was inoculated by activated sludge mixed in a 1:1 ratio with raw reject water. The TAN concentration of the mixture reached 644 mg L^{-1} at the moment of the treatment process initiation. VSS concentration decreased gradually from the starting value reaching 2.7 g L^{-1}, during the first month of reactor operation. Subsequently, an average VSS of 0.8 ± 0.2 g L^{-1} was measured within the remaining operational period. Average NLR of 0.19 ± 0.04 kg N $(m^3\ d)^{-1}$ was similar to the CSTR 1. Since day 10 onwards, NOE achieved was $51.3 \pm 3.7\%$ and NOR was 0.10 ± 0.02 kg N $(m^3\ d)^{-1}$ (Fig. 2(B)).

Likewise the CSTR 1, nitritation was initiated significantly faster compared to nitratation (after 9 and 51 d, respectively), whereas FA and consequently also FNA were the main inhibiting factors for NOB and AOB (Fig. 2(C)). However, both processes started considerably earlier in the case of CSTR 2 compared to CSTR 1. This observation indicates that not even FA- and FNA-acclimatized AOB and NOB groups were able to tolerate FA and/or FNA concentrations exceeding the inhibition levels stated in the literature [11,12] after a certain adaptation period. Such observation is in accordance with the literature [21,33,34].

The development in both the reactors (CSTR 1 and CSTR 2) clearly demonstrates that CSTR systems under the given conditions cannot be operated in shortcut nitrification mode on a long-term basis using FA and/or FNA as the sole factor inhibiting NOB activity. Even though shortcut nitrification induced by a strong initial impulse (such as excessive FA concentration at the moment of reactor initiation) could be stable for a considerable time period, it eventually switched to full nitrification to nitrates. This finding is in accordance with Svehla et al. [16].

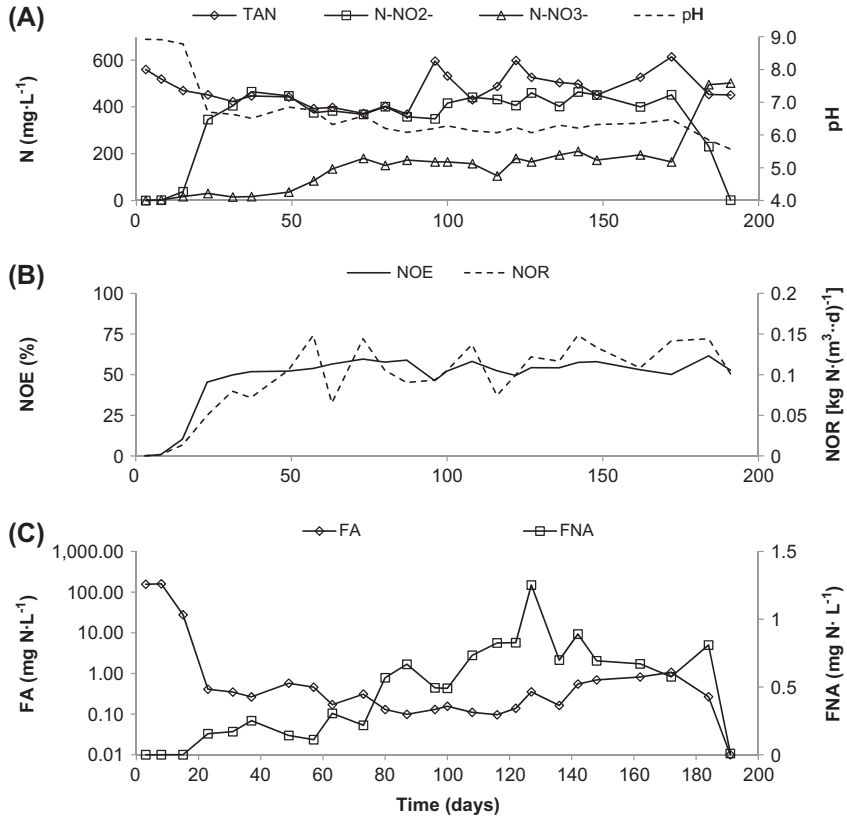

Fig. 1. (A) Nitrogen species and pH values in the CSTR 1, (B) NOE and nitrogen oxidation rate (NOR) in the CSTR 1, and (C) Concentrations of FA and FNA in the CSTR 1 (FA—*Y*-axis exponential).

3.3. Start-up process in CSTR with inoculum and low initial TAN concentration

Contrary to previous models, the initial TAN concentration in CSTR 3 was negligible—basically the equivalent to the concentration in the activated sludge, which was ~0.2 mg L^{-1}. Nitrification activity was detected immediately after initiating the treatment process in the reactor. The day after initiation, 165 mg L^{-1} of $N\text{-}NO_3^-$ was observed, and after another four days, an increase to 430 mg L^{-1} was detected (Fig. 3(A)). Simultaneously, relatively low pH value (approx. 6.3–6.7) was measured during the first five days of the experiment (Fig. 3(A)). Consequently, the pH ranged between 5.6 and 6.0 throughout the remainder of the operational period. Low pH values together with low starting TAN concentrations led to FA concentrations not exceeding 0.001 mg N L^{-1} at the moment of treatment process initiation. The subsequent gradual increase of TAN concentrations up to 600 mg N L^{-1} did not result in strong FA concentration increase (maximum FA 1.56 mg N L^{-1}, Fig. 3(C)) at such low pH value. Moreover, due to minimal $N\text{-}NO_2^-$ accumulation, no significant inhibition effect of FNA (0.03 mg N L^{-1} at maximum) was registered during the experiment, despite the decline in pH value discussed above (Fig. 3(A)). Even though the FA concentration recorded in the reactor could be inhibiting for NOB, according to the literature [11,12], the activity of nitrifying biomass was not affected throughout the entire operational period under the given conditions. As a consequence, even though the average NLR in CSTR 3 (0.50 ± 0.05 kg N $(m^3\ d)^{-1}$) was considerably higher than in CSTR 1 and CSTR 2, AOB as well as NOB were fully active and intensive nitrification with $N\text{-}NO_3^-$, as the main final product was registered from the beginning of the reactor operation. At an average NOE 51.2 ± 3.8 NOR reached 0.25 ± 0.05 kg TAN $(m^3\ d)^{-1}$ (Fig. 3(B)). Results proved that the described reactor start-up strategy is feasible for instant initiation of nitrification even at input TAN concentration exceeding 1,000 mg N L^{-1}.

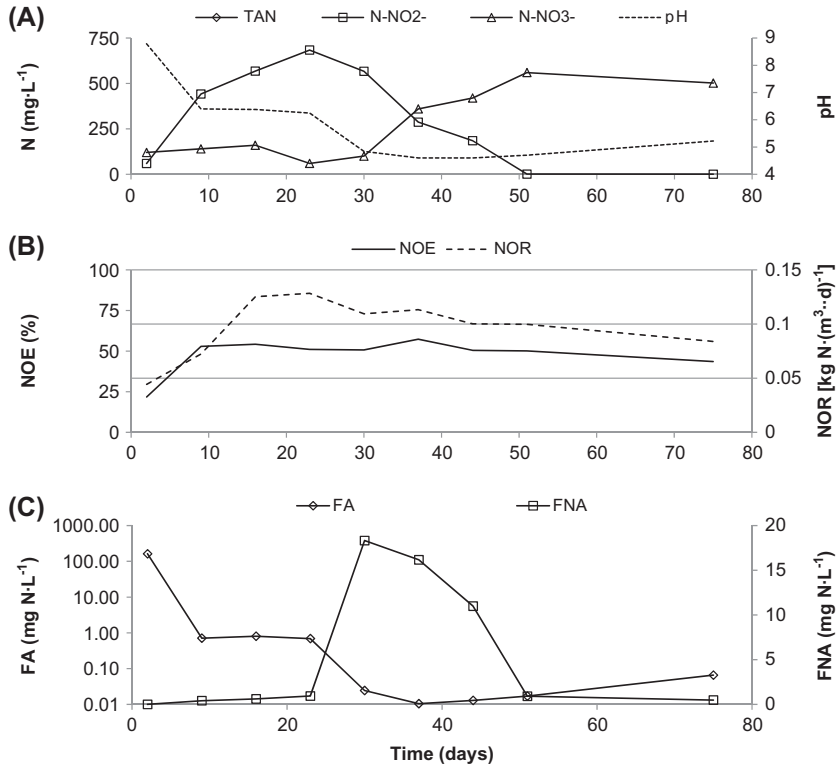

Fig. 2. (A) Nitrogen species and pH values in CSTR 2, (B) NOE and nitrogen oxidation rate (NOR) in the CSTR 2, and (C) Concentrations of FA and FNA in CSTR 2 (FA—*Y*-axis exponential).

3.4. Start-up process in SBR system

As with CSTR 3, full nitrification occured from the beginning of the SBR operation (Fig. 4(A)). As a result of the acidification induced by the full nitrification process [18], significant fluctuations of pH, and consequently also FA concentrations were initiated within SBR cycles in accordance with the system applying shortcut nitrification [16]. $N\text{-}NO_3^-$ concentrations increased to 136 mg L^{-1} during the first day of reactor operation, reaching a maximum of 638 mg L^{-1} on day 7. $N\text{-}NO_2^-$ concentration did not exceed 1 mg L^{-1} until day 12. Even though the FA concentration at the beginning of the SBR cycle reached 3.97 mg N L^{-1} in this phase of the reactor operation and significantly exceeded NOB inhibition limits [10,12], the reactor was operated as a full nitrification system. The actual boundary of FA concentration for efficient inhibition therefore has to be higher under the given conditions. A significant change in the representation of nitrification products was evident after day 14, when the NLR was increased from 0.3 to 0.5 kg TAN $(m^3\ d)^{-1}$. As a consequence, AOB were, for a short time, not able to nitrify TAN effectively due to the temporary overloading of the system, which is apparent from a short-term fall of NOE from 42.1 to 25.8%, observed on day 15 (Fig. 4(B)). NOR remained stable (0.13 kg TAN $(m^3\ d)^{-1}$) between the days 12 and 15, despite an NLR increase detected on day 14. A significant increase in pH, up to 8.4, was observed even at the end of the cycle due to a decrease in H^+ production [18] (Fig. 4(A)). The increase in pH resulted in high FA concentration throughout the entire cycle (up to 89 mg N L^{-1} on day 15) and the decrease in NOB activity was thus accompanied with an increase in $N\text{-}NO_2^-$ concentration (15 mg L^{-1} measured on day 15 and 465 mg L^{-1} detected four days later). Under these conditions, after the restoration of the pH fluctuation during the SBR cycle observed at day 19, the NOB biomass was exposed to alternating inhibition effects of FA and FNA during the SBR cycle. Although the maximum pH value during the cycle (which occurred at the beginning of the cycle), and simultaneously also the maximum FA concentration, gradually fell to original levels between days 15 and 19, the shortcut nitrification remained stable until the end of the reactor operation (day 22). This is evidenced by the gradual decrease in $N\text{-}NO_3^-$ concentration and simultaneous increase in $N\text{-}NO_2^-$ concentration from

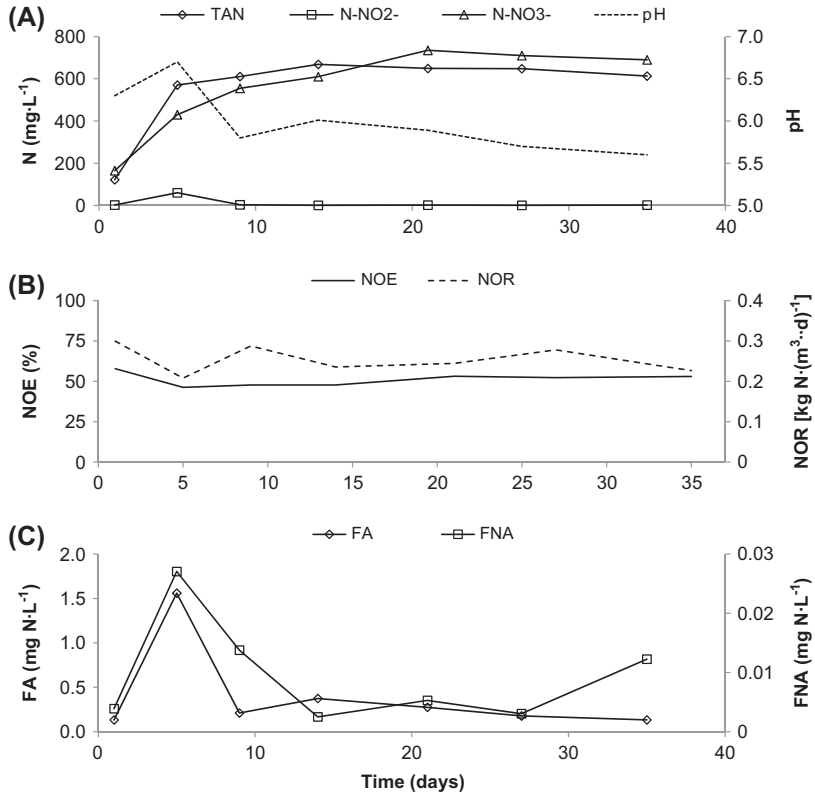

Fig. 3. (A) Nitrogen species and pH values in CSTR 3, (B) NOE and nitrogen oxidation rate (NOR) in the CSTR 3, and (C) Concentrations of FA and FNA in CSTR 3.

day 19 onwards. The FA concentration (at the cycle beginning) and the FNA concentration (at the cycle end) reached 6.9 and 1.85 mg N L^{-1}, respectively at the end of the experiment (day 22) (Fig. 4(C)). These values are able to permanently restrict NOB activity in SBR under given conditions as was proved by Svehla et al. [16].

As a consequence of gradual decrease in treatment process intensity during the cycle, certain increase in DO concentration was observed during the working phase of the cycle. The concentration of DO reached an average of 5.2 ± 1.1 and 2.4 ± 0.7 mg L^{-1} at the beginning and at the end of working phase, respectively.

3.5. Application of results for the accelerated start-up of shortcut nitrification

Based on the presented results (CSTR 3 and SBR), it could be concluded that immediate initiation of full nitrification for treatment of extremely high nitrogen-loaded wastewater is completely feasible using common activated sludge from municipal WWTP as a sole inoculum, in the case that very simple control of environmental conditions (especially FA and FNA concentration) during the start-up phase is applied. This approach could be used not only for separate treatment of reject water, but also for landfill leachate or specific industrial wastewaters with similar characteristics. The results of presented experiment seem to be very promising because earlier studies indicate that even in a case, when a special strategy for the acceleration of start-up process is applied, the period lasting for several tens of days is necessary for a satisfactory initiation of the process [26,27].

The reliable method for an efficient start-up of shortcut nitrification during the treatment of high-strength wastewater consists of an immediate start-up of full nitrification and its consequent transformation into the shortcut nitrification mode. Temporary increase in FA concentration could be used for this purpose (see results of the operation of SBR). Also permanent or temporary oxygen limitation could have similar effect [7,8]. Consequently, in the case of the SBR the rapid fluctuation of FA and FNA concentrations is able to ensure permanent NOB inhibition [16]. The concentration of TAN in treated water is a very

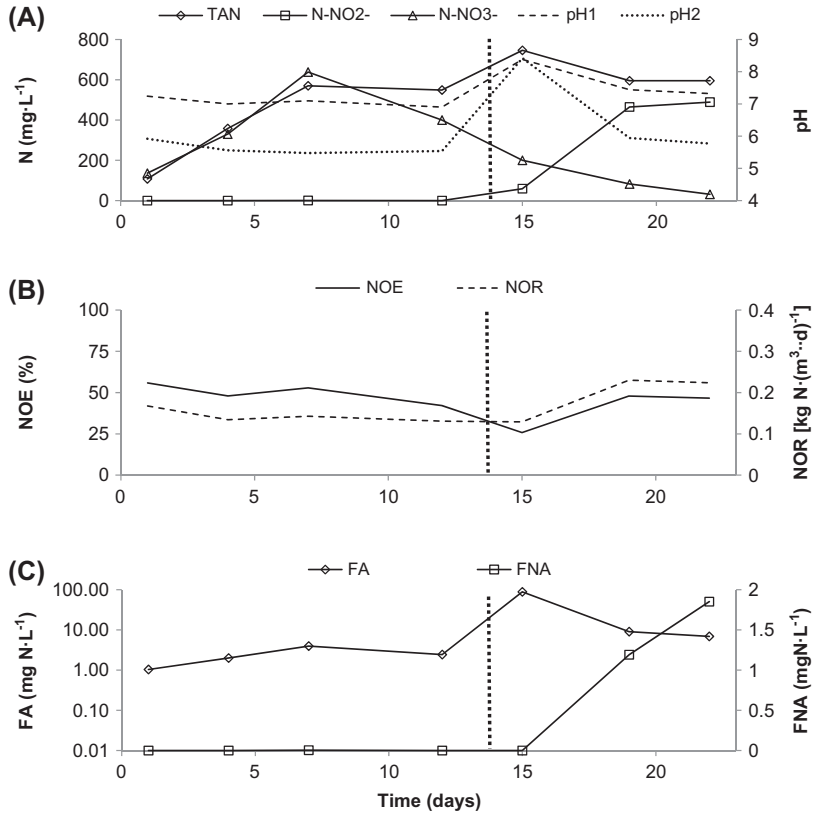

Fig. 4. (A) Nitrogen species and pH values in the SBR, (dashed line indicates the increase of NLR from 0.3 to 0.5 kg TAN $(m^3\ d)^{-1}$, (B) NOE and nitrogen oxidation rate (NOR) in the CSTR 3, and (C) Maximum FA and FNA concentrations in the SBR. FA values measured at the beginning of the cycle, FNA values at the end of the cycle (FA—*Y*-axis exponential).

important factor influencing actual inhibition pressure of FA and/or FNA toward nitrifying biomass, as is the availability of shortcut nitrification in SBR systems [35,36]. Svehla et al. [36] proved that FA concentration reaching 5.8 mg N L^{-1} at the beginning of SBR cycle combined with FNA concentration reaching ca. 0.3 mg N L^{-1} at the end of the cycle is able to effectively inhibit NOB during shortcut nitrification of diluted reject water in SBR. However, the strategy using FA and/or FNA inhibition as the sole factor for the achievement of shortcut nitrification is not feasible in CSTR systems [11,16]. Here, it is necessary to combine the effect of FA and/or FNA with an additional inhibition pressure on NOB, such as DO limitation with the aim of ensuring shortcut nitrification on a long-term basis [8].

Jenicek et al. [17] proved that the system applying shortcut nitrification in SBR under conditions analogical with the SBR system presented in this paper is able to ensure NOR exceeding 1.5 kg N $(m^3\ d)^{-1}$. Similarly, Pacek et al. [8] reached NOR exceeding 1.1 kg N $(m^3\ d)^{-1}$ in a system applying DO control in CSTR with the aim to minimize NOB activity. It was proved that DO limitation may decrease the productivity of tested system [8]. At all events, both systems (SBR and CSTR) show productivity at least comparable with the SHARON process operated usually at an NLR lower than 1 kg N $(m^3\ d)^{-1}$ and simultaneously at temperatures significantly exceeding the value applied in our experiments [37]. The results of this paper indicate that such productive systems could be initiated practically immediately. A quick start-up of the treating process would also enable the possibility to operate the process seasonally, if needed, e.g. to treat reject water only in the periods with nitrogen concentration exceeding emission limits in the effluent from WWTP.

NOE, in all four operated experimental reactors, was limited to an average 50.5 ± 3.0% by an insufficient ratio between alkalinity and TAN concentration in treated reject water even in the case that nitrification (or at least nitritation) was satisfactorily established in the system [38]. In order to evaluate possible differences between NOE in differently initiated reactors exposed to various NLR, the NOE values

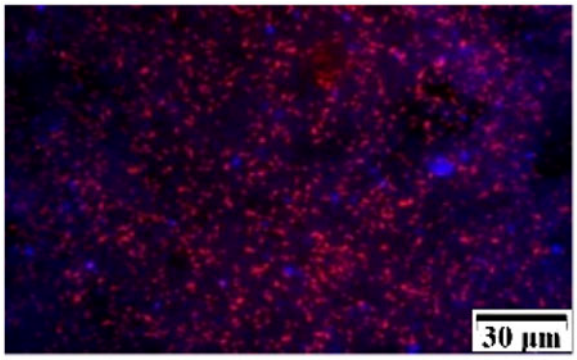

Fig. 5. FISH analysis of raw reject water, AOB red colored, enlargement 320×).

measured in all four experimental reactors were statistically evaluated by a Kruskal–Wallis test. The *p*-values for all compared variants were higher than the significance level of α (0.05). No statistical differences between the variants were observed. Such range of NOE (approx. 50% from) is typical for successful nitrification (as well as for shortcut nitrification) of reject water without pH control [3,17]. The dosing of alkali could be used for the improvement of NOE in the system applying nitrification/denitrification or shortcut nitrification/denitrification [17]. On the other hand, achieved values of NOE lead to $N\text{-}NO_2^-$/TAN ratio suitable for subsequent ANAMMOX process in the case that NOB are effectively washed out from a nitrifying system [3].

3.6. The influence of temperature

In case the above-mentioned results would be applied to the operation of full-scale reactors, temperature will be an important factor influencing the treatment process due to strong temperature dependence of nitrification rate [18]. The experiments were performed at the laboratory temperatures (23 ± 2˚C), which is relatively high compared to the real temperature of municipal wastewater, especially in winter seasons. On the other side, 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. This could be beneficial for achievement of sufficient temperatures within the full-scale application of results presented within this paper. In addition, Hrncirova et al. [39] proved that the system applying shortcut nitrification under technological conditions comparable with this study is able to be satisfactorily operated at strongly fluctuating temperatures. According to their results, the sufficient conversion of TAN into $N\text{-}NO_2^-$ was achieved from a long-term perspective even at 13˚C. Therefore, also the possibility to apply the results to cold wastewater streams cannot be excluded. In all events, comparing with current systems for biological treatment of extremely high nitrogen-loaded wastewaters (e.g. SHARON or ANAMMOX processes operated usually at 30–40˚C [2,9]) the temperatures applied in our experiment seems to be rather low.

3.7. *Fluorescence* in situ *hybridization*

Quantitative FISH analyses using three samples for each analyzed material were performed with raw reject water, samples from CSTR 1, and CSTR 3 on days 50 (Fig. 6(A)) and 30 (Fig. 6(B)), respectively. The relative abundance of each bacterial strain was determined in triplicate. The result determined a percentage expression of the area occupied by nitrifying bacteria in related to the area of total biomass. The content of AOB biomass in a sample from reject water with TSS 1.09 g L^{-1} reached 2.4 ± 0.7% from the total

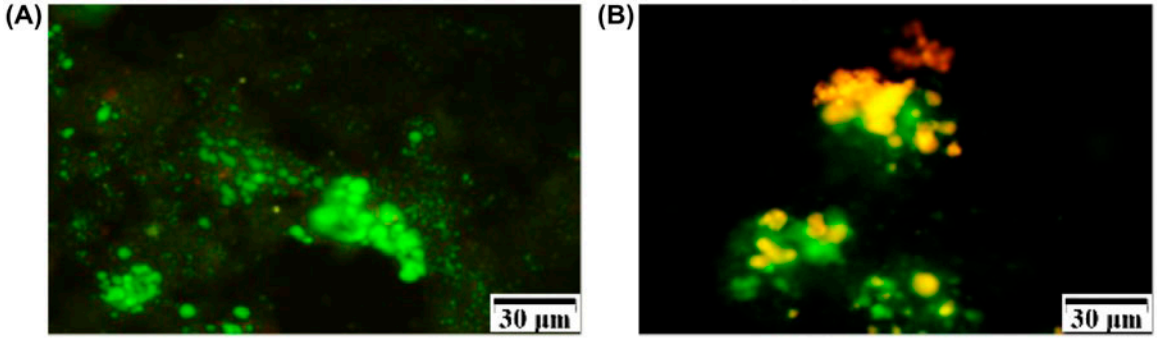

Fig. 6. FISH analyses in the CSTRs 1 and 3, (A) CSTR 1 sample and (B) CSTR 3 sample—AOB green colored, yellow-red NOB, enlargement 320×).

biomass (Fig. 5). This finding is in accordance with the results of chemical analysis indicating relatively fast initiation of AOB activity. However, the amount of NOB was below detectable limit (0.1%) in all measurements.

The sample taken from CSTR 1 shows significant predominance of AOB (13.1 ± 1.9%) over NOB (below 1%) from the total biomass (Fig. 6(A)), yet this amount was still sufficient to convert more than one-third of the total oxidized nitrogen into $N\text{-}NO_3^-$. The sample taken from CSTR 3 documents relatively equal amount of AOB and NOB (8.1 ± 1.1% and 7.8 ± 1.2% from the total biomass, respectively), which is in agreement with the full nitrification in CSTR 3 (Fig. 6(B)).

4. Conclusion

Raw reject water contains a certain amount of AOB, making it possible to start-up the reactor applying shortcut nitrification for reject water treatment even without the addition of any inoculum. Inoculation with common activated sludge significantly accelerates the initiation of AOB as well as NOB activity. However, in the case that high initial TAN concentration is applied, mainly the NOB activity could be strongly inhibited by elevated FA concentration for a certain period. The immediate initiation of full nitrification with nitrate as a dominant final product was achieved in the case of zero initial TAN concentration in CSTR as well as in SBR at NLR reaching ca. 0.5 and 0.3 kg N $(m^3\ d)^{-1}$, respectively. High AOB and also NOB activity were monitored during the first day of experiments under such conditions despite high input TAN concentration exceeding 1 g L^{-1}. Fast and simple conversion of traditional full to shortcut nitrification with nitrite as main final product was achieved by a strong short-term impulse consisting in the temporary increase in FA concentration up to 89 mg N L^{-1}. Consequently, the fluctuation in FA and FNA concentrations has the ability to restrict NOB activity permanently in SBR. On the other hand, in CSTR the inhibition effect of FA and/or FNA is necessary to combine with other factor(s) limiting NOB activity such as DO limitation for this purpose.

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References

[1] J. Jeavons, L. Stokes, J. Upton, M. Bingley, Successful sidestream nitrification of digested sludge liquors, Water Sci. Technol. 38 (1998) 111–118.
[2] S. Lackner, E.M. Gilbert, S.E. Vlaeminck, A. Joss, H. Horn, M.C.M. van Loosdrecht, Full-scale partial nitritation/anammox experiences—An application survey, Water Res. 55 (2014) 292–303.
[3] U. van Dongen, M.S.M. Jetten, M.C. van Loosdrecht, The SHARON-Anammox process for treatment of ammonium rich wastewater, Water Sci. Technol. 44 (12) (2001) 153–160.
[4] J.A. Torà, J. Lafuente, C. Garcia-Belinchón, L. Bouchy, J. Carrera, J. Baeza, High-throughput nitritation of reject water with a novel ammonium control loop: Stable effluent generation for anammox or heterotrophic denitritation, Chem. Eng. J. 243 (2014) 265–271.
[5] I. Zekker, E. Rikmann, T. Tenno, A. Seiman, L. Loorits, K. Kroon, M. Tomingas, P. Vabamäe, T. Tenno, Nitritating-anammox biomass tolerant to high dissolved oxygen concentration and C/N ratio in treatment of yeast factory wastewater, Environ. Technol. 35(12) (2014) 1565–1576.
[6] I. Zekker, E. Rikmann, T. Tenno, L. Loorits, K. Kroon, P. Vabamäe, A. Mandel, M. Raudkivi, T. Tenno, Nitric oxide for anammox recovery in nitrite-inhibited deammonification system, Environ. Technol. 36(19) (2015) 2477–2487.
[7] A. Guisasola, I. Jubany, J.A. Baeza, J. Carrera, H. Horn, J. Lafuente, Respirometric estimation of the oxygen affinity constants for biological ammonium and nitrite oxidation, J. Chem. Technol. Biotechnol. 80 (2005) 388–396.
[8] L. Pacek, P. Švehla, J. Bartáček, J. Radechovský, H. Hrnčířová, Š. Shejbalová, J. Balík, P. Jeníček, Direct and indirect effects of oxygen limitation on nitrification process applied to reject water treatment, Desalin. Water Treat. (2014), doi: 10.1080/19443994.2014.950336.
[9] C. Hellinga, A.A.J.C. Schellen, J.W. Mulder, M.C.M. van Loosdrecht, J.J. Heijnen, The sharon process: An innovative method for nitrogen removal from ammonium-rich waste water, Water Sci. Technol. 37 (1998) 135–142.
[10] D.J. Kim, D.I. Lee, J. Keller, 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 (2006) 459–468.
[11] S. Park, W. Bae, Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid, Process Biochem. 44 (2009) 631–640.
[12] A.C. Anthonisen, R.C. Loehr, T.B.S. Prakasam, E.G. Srinath, Inhibition of nitrification by ammonia and nitrous acid, J. Water Pollut. Control Fed. 48 (1976) 835–852.
[13] V.M. Vadivelu, J. Keller, Z. Yuan, Effect of free ammonia on the respiration and growth processes of an enriched *Nitrobacter* culture, Water Res. 41 (2007) 826–834.
[14] V.M. Vadivelu, J. Keller, Z. Yuan, Effect of free ammonia and free nitrous acid concentration on the anabolic and catabolic processes of an enriched *Nitrosomonas* culture, Biotechnol. Bioeng. 95 (2006) 830–839.

[15] Q. Wang, L. Ye, G. Jiang, S. Hu, Z. Yuan, Side stream sludge treatment using free nitrous acid selectively eliminates nitrite oxidizing bacteria and achieves the nitrite pathway, Water Res. 55 (2014) 245–255.
[16] P. Svehla, J. Bartacek, L. Pacek, H. Hrncirova, J. Radechovsky, A. Hanc, P. Jenicek, Inhibition effect of free ammonia and free nitrous acid on nitrite-oxidising bacteria during sludge liquor treatment: Influence of feeding strategy, Chem. Pap. 68 (2014) 871–878.
[17] P. Jenicek, P. Svehla, J. Zabranska, M. Dohanyos, Factors affecting nitrogen removal by nitritation/denitritation, Water Sci. Technol. 49 (2004) 73–79.
[18] M. Henze, M.C.M. van Loosdrecht, G. Ekama, D. Brdjanovic, Biological Wastewater Treatment: Principles, Modelling and Design, IWA Publishing, London, 2008.
[19] Y. Cohen, H. Kirchmann, Increasing the pH of wastewater to high levels with different gases—CO_2 stripping, Water Air Soil Pollut. 159 (2004) 265–275.
[20] M.H. Gerardi, Wastewater Microbiology: Nitrification/Denitrification in the Activated Sludge Process, John Wiley and Sons, Inc., New York, NY, 2002.
[21] Y. Zhou, A. Oehmen, M. Lim, V. Vadivelu, W.J. Ng, The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants, Water Res. 45 (2011) 4672–4682.
[22] M. Vallés-Morales, J. Mendoza-Roca, A. Bes-Pia, A. Iborra-Clar, Nitrogen removal from sludge water with SBR process: start-up of a full-scale plant in the municipal wastewater treatment plant at Ingolstadt, Germany, Water Sci. Technol. 50 (2004) 51–58.
[23] S. van Hulle, S. Van Den Broeck, J. Maertens, K. Villez, B. Donckels, G. Schelstraete, E. Volcke, P. Vanrolleghem, Construction, start-up and operation of a continuously aerated laboratory scale SHARON reactor in view of coupling with an Anammox reactor, Water SA 31 (2005) 327–334.
[24] J. Zhang, J. Zhou, Y. Han, X. Zhang, Start-up and bacterial communities of single-stage nitrogen removal using anammox and partial nitritation (SNAP) for treatment of high strength ammonia wastewater, Bioresour. Technol. 169 (2014) 652–657.
[25] I. Zekker, E. Rikmann, L. Loorits, T. Tenno, H. Fritze, T. Tuomivirta, K. Kroon, P. Vabamäe, A. Seiman, M. Raudkivi, A. Mandel, T. Tenno, Start-up of low temperature anammox in UASB from mesophilic yeast factory anaerobic tank inoculum, Environ. Technol. 36 (2) (2014) 214–225.
[26] I. Jubany, J. Carrera, J. Lafuente, J.A. Baeza, Start-up of a nitrification system with automatic control to treat highly concentrated ammonium wastewater: Experimental results and modeling, Chem. Eng. J. 144 (2008) 407–419.
[27] A. Bartrolí, J. Carrera, J. Pérez, Bioaugmentation as a tool for improving the start-up and stability of a pilot-scale partial nitrification biofilm airlift reactor, Bioresour. Technol. 102 (2011) 4370–4375.
[28] S. Sri Shalini, K. Joseph, Start-up of the SHARON and ANAMMOX process in landfill bioreactors using aerobic and anaerobic ammonium oxidising biomass, Bioresour. Technol. 149 (2013) 474–485.
[29] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, twentieth edition, APHA, Washington, DC, (1998).
[30] H. Daims, S. Lücker, M. Wagner, Daime, a novel image analysis program for microbial ecology and biofilm research, Environ. Microbiol. 8 (2006) 200–213.
[31] U. Abeling, C.F. Seyfried, Anaerobic-aerobic treatment of high strength ammonium wastewater-nitrogen removal via nitrite, Water Sci. Technol. 26(5–6) (1992) 1007–1015.
[32] C. Fux, M. Boehler, P. Huber, I. Brunner, H. Siegrist, Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant, J. Biotechnol. 99 (2002) 295–306.
[33] O. Turk, D.S. Mavinic, Stability of nitrite build-up in an activated sludge system, J. Water Pollut. Control Fed. 61 (1989) 1440–1448.
[34] C. Rongsayamanont, T. Limpiyakorn, B. Law, E. Khan, Relationship between respirometric activity and community of entrapped nitrifying bacteria: Implications for partial nitrification, Enzyme Microb. Technol. 46 (2010) 229–236.
[35] V. Kouba, M. Catrysse, H. Stryjova, I. Jonatova, E. Volcke, P. Svehla, J. Bartacek, The impact of influent total ammonium nitrogen concentration on nitrite-oxidizing bacteria inhibition in moving bed biofilm reactor, Water Sci. Technol. 69(6) (2014) 1227–1233.
[36] P. Svehla, J. Radechovsky, H. Hrncirova, L. Pacek, J. Bartacek, Effect of influent nitrogen concentration on feasibility of short-cut nitrification during wastewater treatment in activated sludge systems, Chem. Pap. 69 (7) (2015) 921–929.
[37] R. van Kempen, J.W. Mulder, C.A. Uijterlinde, M.C.M. Loosdrecht, Overview: Full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering, Water Sci. Technol. 44(1) (2001) 145–152.
[38] T. Khin, A.P. Annachhatre, Novel microbial nitrogen removal processes, Biotechnol. Adv. 22 (2004) 519–532.
[39] H. Hrncirova, P. Svehla, J. Radechovsky, L. Pacek, J. Balik, The influence of temperature fluctuation on the stability of short-cut nitrification applied for reject water treatment, Environ. Prot. Eng. (in press).