

HELENA RADECHOVSKA<sup>1</sup>, PAVEL SVEHLA<sup>1</sup>,  
JOSEF RADECHOVSKY<sup>1</sup>, LUKAS PACEK<sup>1</sup>, JIRI BALIK<sup>1</sup>

## THE INFLUENCE OF TEMPERATURE FLUCTUATION ON THE STABILITY OF PARTIAL NITRITATION APPLIED FOR REJECT WATER TREATMENT

The influence of sudden and gradual temperature fluctuations on the stability of partial nitrification applied for the treatment of reject water containing ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) in the range of 1000–1400  $\text{mg/dm}^3$  was investigated. It was found that a sudden decrease in temperature from 24.3 °C to 14.3 °C and 15.8 °C significantly affected the stability of the process. No negative effect was recorded after a sudden temperature decrease from 24.3 to 17.4 °C. It was also found that during a gradual temperature decrease from 24.3 °C, process stability was negatively affected at 12.7 °C.

### 1. INTRODUCTION

Nitrogen removal during wastewater treatment is traditionally ensured by the biological process of nitrification/denitrification. In some cases, the application of this method is complicated; the treatment of effluent with a low concentration ratio of COD:N is especially problematic. Multiple innovative biological methods have been implemented in order to treat these types of wastewater. These methods are based on the accumulation of nitrite during the nitrification process. The procedures include a process of nitrification/denitrification and of partial nitrification combined with anaerobic ammonium oxidation (anammox). Nitrification/denitrification is based on the fact that under specific conditions it is possible to stop N-ammon oxidation by controlling nitrite levels. Under ideal operational conditions, it is possible to reduce up to 25% of the oxygen consumed/needed during nitrification as compared with nitrification, and up to 40%

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<sup>1</sup>Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiolgy, Food and Natural Resources, CULS in Prague, Kamycka 129, Prague 6 – Suchdol, 165 21, e-mail: hrncirovah@af.czu.cz

of organic substrate during denitritation as compared with denitrification, resulting in a faster process and a reduced volume of grown biomass [1, 2].

Another innovative biological method is an anammox process based on a microbial-mediated reaction between nitrite ions produced during nitrification and ammonium ions remaining in the treated water. Elemental nitrogen is generated during this process [3–5]. No organic carbon is needed for the satisfactory operation of the anammox process. Simultaneously, the process requires a theoretical 1:1.32 ratio of  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_2^-\text{-N}$ . Correspondingly, only ca. 60% of  $\text{NH}_4^+\text{-N}$  should theoretically be converted to  $\text{NO}_2^-\text{-N}$ , meaning partial nitrification is needed as a first stage for subsequent anammox [6, 7]. In contrast, for efficient nitrification/denitrification it is necessary to transform all  $\text{NH}_4^+\text{-N}$  into  $\text{NO}_2^-\text{-N}$  before its reduction to dinitrogen gas. Therefore, oxygen demand is significantly lower for partial nitrification/anammox compared to nitrification/denitrification. Both processes could be operated in a one-step setup or in two separated reactors (one for nitrification/partial nitrification and one for denitrification or anammox [8–13]). Reject water arising during the thickening and dewatering of digested sludge, which is characterized by very high  $\text{NH}_4^+\text{-N}$  concentrations, is a typical wastewater suitable for the application of the methods mentioned above [8]. Nitrite accumulation, which is the basis for nitrification and partial nitrification, may be stimulated by intervening with the nitrification system in a way that suppresses nitrite oxidizing bacteria (NOB) while preserving the function of ammonia oxidizing bacteria (AOB). The aim is to get the biomass to a point at which the activity of AOB exceeds the activity of NOB. The main factors that can be used to stop the nitrification on nitrite level are limited concentration of dissolved oxygen [14, 15] or low sludge age [16]. Nitrification or partial nitrification is successfully achieved in the sequencing batch reactor (SBR) thanks to strong fluctuations of free ammonia (FA) and free nitrous acid (FNA) concentrations [9, 17]. These chemical substances selectively inhibit NOB activity in a specific concentration range [18–20].

Temperature is an important factor affecting the nitrification process on a number of counts. Nitrifying organisms are generally very sensitive to temperature; optimum levels lie in the range of 28–32 °C. With a temperature decrease of 10 °C, the nitrification rate decreased by ca. 50% [21]. Higher temperatures facilitate the accumulation of nitrite [22]. The combined effect of high temperature with sludge retention time (SRT) limitation is the principle of the single reactor system for high activity ammonium removal over nitrite (SHARON) process [8]. Current systems of separate biological treatment of reject water based on the accumulation of nitrites are usually operated at a temperature in the range of ca. 30–40 °C [8, 23]. Reject water is characterized by high temperatures, but from a technological point of view it can be difficult to maintain temperatures within the required/ideal range during the winter. Any heating of the reactor significantly deteriorates the economy of the process. Therefore, it seems appropriate to look for technological alternatives that do not require temperature control [24]. The possibility of operating high nitrogen loaded SBR systems treating reject water at relatively

low temperatures has been previously confirmed. Partial nitrification with flocculent biomass was successfully applied at  $23 \pm 2$  °C [17] for this purpose. This value seems to be sustainable throughout most seasons thanks to the high temperature of raw reject water produced during the thickening and dewatering of sludge digested under mesophilic or thermophilic conditions [25]. However, it is necessary to consider the effects of temporary drops below these values during the winter, and the stability of partial nitrification (and full nitrification as well) after significant decreases in temperature may be disrupted in the case of particularly strong fluctuations in temperature.

This work aimed to evaluate partial nitrification temperature resistance during separate reject water treatment applied in a system using flocculent biomass operated on the SBR principle. Based on [17], 25 °C was selected as an adjusted starting temperature value, where the effect of sudden temperature decreases with variable intensity on process stability was assessed. Simultaneously, the determination of the minimal temperature value enabling satisfactory operation of the system was also an aim of this study. Tests simulating slow temperature decreases from an initial adjusted temperature of 25 °C were realized for this purpose.

## 2. EXPERIMENTAL

*Reactor set-up.* A laboratory model of a nitrifying reactor made of plastic (0.75 dm<sup>3</sup> in volume) was operated with flocculent biomass cultivated in the form of activated sludge. The model was placed in a thermostatic box with the aim of ensuring optimal conditions for temperature control. Peristaltic pumps and silicone tubes were used for the transport of treated water into the reactor. The reactor was aerated using the coarse bubble system. It was operated on the SBR principle with two 12 hour cycles per day. Each cycle consisted of the following repeated phases: inflow of treated water into the reactor (ca. 10 min), the working phase, when the reactor volume was aerated (11 h 20 min), sedimentation (ca. 20 min), and drainage of reject water from the reactor (ca. 10 min). Based on the results of previous tests [9, 17], the system described above was selected as a suitable technological arrangement for the effective partial nitrification of reject water. The nitrogen loading rate (NLR) was maintained at 0.2 kg/(m<sup>3</sup>·d). The concentration of dissolved oxygen in the reactor was not limited: at the beginning of the cycle the concentration was 6.1 mg O<sub>2</sub>/dm<sup>3</sup> on average and at the end of the cycle it was 7.7 mg O<sub>2</sub>/dm<sup>3</sup> on average. The pH value was not controlled. The concentration of volatile suspended solids (VSS) ranged between 0.25 and 3 g/dm<sup>3</sup>. The stability of the partial nitrification process after the temperature changes was evaluated based mainly on the changes in pH and representation of nitrogen compounds in the effluent.

*Treated water.* Reject water from the Central Waste Water Treatment Plant in Prague was used. Its quality parameters are shown in Table 1.

Table 1

Composition of reject water

Parameter	Average	Range
pH	8.4	8.1–8.7
Alkalinity, mmol/dm <sup>3</sup>	93	70–121
P total, mg P/dm <sup>3</sup>	37	23–63
NH <sub>4</sub> <sup>+</sup> -N, mg NH <sub>4</sub> <sup>+</sup> /d	1336	985–1734
COD, mg O <sub>2</sub> /dm <sup>3</sup>	2700	1145–4600
COD soluble, mg O <sub>2</sub> /dm <sup>3</sup>	1532	770–2322
TSS, g/dm <sup>3</sup>	3.3	2.1–5.5

*The method of temperature changes.* The laboratory model described above was operated for 392 days. The operational time was divided into 8 periods (1–8). A schedule of temperature changes during the experiment is shown in Table 2.

Table 2

Schedule of changes of temperature during the experiment

Operational period	Days	Adjusted temperature [°C]	Real temperature [°C]	
			Range	Average
1	0–13	25	24–24.7	24.3
2	14–46	15	14.1–14.5	14.3
3	47–171	25	24–24.7	24.3
4	172–192	18	17.1–17.6	17.4
5	193–228	25	24–24.7	24.3
6	229–276	16.5	15.5–15.9	15.8
7	277–360	25	24–24.7	24.3
8	361–392	(25→13)	12.7–24.3	–
	361	23	–	22.4
	362	21	–	20.3
	363	19	–	18.4
	364–371	17	16.2–16.4	16.3
	372–375	15	14.2–14.6	14.4
	376–392	13	12.5–12.9	12.7

During this time the temperature in the system was suddenly changed several times (operational periods 1–7). The value of 25 °C was selected as an initial adjusted temperature (period 1) in accordance with the conditions applied within previous tests [17]. During periods 2, 4, and 6, sudden drops in temperature with variable intensities (one by one to 15, 18 and 16.5 °C) were simulated with the aim of determining the maximum

intensity of temperature decrease the system is able to accept without significantly decreasing the stability of the treatment process under the given conditions. Within periods 3, 5 and 7, the temperature was restored to the original value (25 °C) with the aim of stabilising the process at this temperature before another simulated drop. In the last period of the experiment (period 8, days 361–392), the temperature was decreased gradually with the aim of determining the minimum temperature enabling satisfactory operation of the tested system under the given conditions.

The duration of particular periods was selected with respect to the stability of the treatment process before changes to the temperature regime. Therefore, significant differences can be recorded between the duration of particular periods. Period 1 lasted only 13 days due to the fact that the system was operated under comparable conditions for a period of one month before the initiation of period 1. The only difference was that temperature was based on actual temperature in the laboratory (23±2 °C) instead of artificial control in the thermostatic box. The standard duration of individual periods was around 1 month. However, in cases where significant disruption to process stability was recorded after a radical fall in temperature during particular periods, the operational time was extended (e.g. period 6). Simultaneously, the operational time within periods with the initial temperature of 25 °C (periods 3 and 7) was significantly extended in cases where process stability was disrupted by radical temperature falls within the previous periods with the aim of stabilizing the intensity and performance of the treatment process to levels comparable with period 1.

Real temperature values measured in the reactor differed to a certain extent from adjusted values in the thermostatic box. Simultaneously, the results proved that relatively minimal differences between actual values of temperature may result in significant changes to process stability. Therefore, real average temperature values, with accuracy to tenths of °C, are presented throughout the whole text of this paper (Table 2).

*Analytical methods.* The spectrophotometric measurement of various forms of nitrogen ( $\text{mg NH}_4^+/\text{dm}^3$ ,  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N) and chemical oxygen demand (COD) was performed using a HACH (Hach Lange GmbH, Germany) DR/4000 photometer, and total suspended solids (TSS) and volatile suspended solids (VSS) were determined gravimetrically. All measurements were performed according to Standard Methods [26]. P-total concentration was determined with a HACH DR/4000 photometer by HACH method No. 8190. Alkalinity was determined by titration of the sample with hydrochloric acid (0.1 mol/dm<sup>3</sup>) up to pH 4.5. Temperature, pH and dissolved oxygen concentration were measured by WTW instruments pH 340i and oxi 340i (WTW Wissenschaftlich-Technische Werkstätten GmbH, Germany). The pH value was continuously monitored using a GRYF sensor PCI 321 (GRYF HB, Czech Republic).

*Calculations.* The concentrations of FA ( $C_{\text{FA}}$ ) and FNA ( $C_{\text{FNA}}$ ) were calculated in accordance with Anthonisen et al. [18] and Park and Bae [27]:

$$C_{\text{FA}} = \frac{17}{14} \frac{10^{\text{pH}} C_{\text{NH}_4^+-\text{N}}}{\exp\left(\frac{6334}{273+T}\right) + 10^{\text{pH}}} \left[ \text{mg /dm}^3 \text{ NH}_4^+-\text{N} \right] \quad (1)$$

$$C_{\text{FNA}} = \frac{47}{14} \frac{C_{\text{NO}_2^--\text{N}}}{\left( \exp\left(-\frac{2300}{273+T}\right) + 10^{\text{pH}} \right) + 1} \left[ \text{mg/dm}^3 \text{ HNO}_2 \right] \quad (2)$$

where  $C_{\text{NH}_4^+-\text{N}}$  and  $C_{\text{NO}_2^--\text{N}}$  represent the actual total concentrations of  $\text{NH}_4^+-\text{N}$  and  $\text{NO}_2^--\text{N}$ , respectively, and  $T$  is the temperature in centigrade scale.

Representations of  $[\text{NO}_2^--\text{N}]$  and  $[\text{NO}_3^--\text{N}]$  (%) were calculated using  $C_{\text{NO}_2^--\text{N}}$  and  $C_{\text{NO}_3^--\text{N}}$  concentration in  $\text{mg/dm}^3$

$$[\text{NO}_2^--\text{N}] = 100 \frac{C_{\text{NO}_2^--\text{N}}}{C_{\text{NO}_2^--\text{N}} + C_{\text{NO}_3^--\text{N}}} \quad (3)$$

$$[\text{NO}_3^--\text{N}] = 100 \frac{C_{\text{NO}_3^--\text{N}}}{C_{\text{NO}_2^--\text{N}} + C_{\text{NO}_3^--\text{N}}} \quad (4)$$

### 3. RESULTS AND DISCUSSION

*Evaluation of process stability according to pH value.* The pH value is a good indicator of short-cut nitrification process stability under the conditions applied within the described experiment. The activated sludge mixture is acidified as a consequence of AOB activity during the nitrification process when applied to high nitrogen loaded wastewater [21]. As a consequence, significant pH fluctuations can be recorded during the operational cycle of our SBR system treating reject water in cases where stable partial nitrification took place in this system in accordance with [17].

During the operation of the reactor at 24.3 °C (operational periods 1, 3, 5 and 7), pH in the reactor ranged between 6.9 and 7.9 at the beginning of the SBR cycle and between 4.7 and 6.5 at its end (Fig. 1). These relatively large differences indicate intensive AOB activity and stable partial nitrification. However, after a sudden decrease in temperature from 24.3 °C to 14.3 °C (day 14, operational period 2), a rapid increase in pH to 8.9 was monitored, while no significant differences in pH were recorded during the SBR cycle between days 21 and 41. As late as day 42 (28 days after the start of

period 2), pH at the beginning and at the end of the cycle started to differ again. This indicates a disruption to partial nitrification stability and its gradual return to the original intensity during period 2. A similar development related to pH was recorded during operational period 6 after a decrease in temperature to 15.8 °C. After a decrease in temperature from 24.3 °C to 17.4 °C (operational period 4), this effect was not observed (Fig. 1). During the gradual decrease in temperature from 24.3 °C (operational period 8), the difference between pH at the beginning and at the end of the cycle was reduced with a falling temperature. However, significant differences between the start and end of the cycle were monitored until a temperature of 12.7 °C was reached. Under these conditions, on day 392 pH ranged between 8.8 and 8.9 throughout the entire day, indicating a disruption to process stability. Therefore, no additional decreases in temperature were realized within phase 8 and the experiment was terminated.

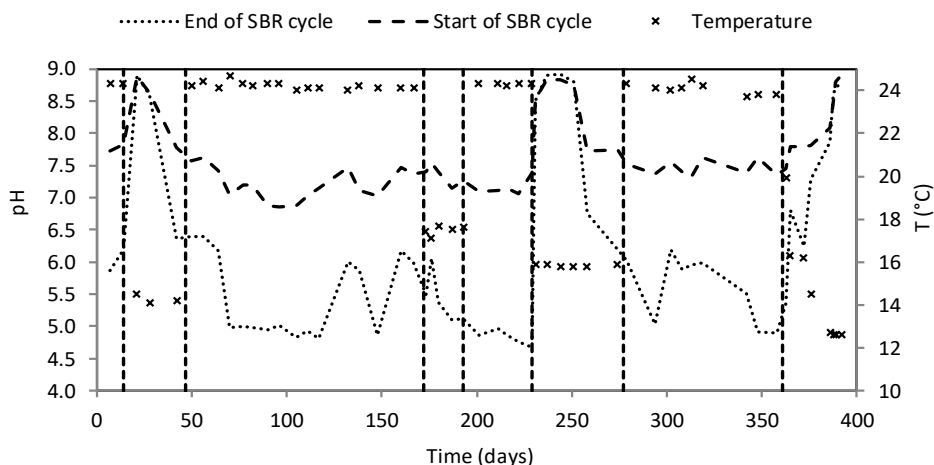


Fig. 1. pH at the beginning and at the end of the SBR cycle during operational periods 1–8

*Evaluation of process stability according to the concentrations of nitrogen compounds in the effluent.*  $\text{NO}_2^-$ -N is the final product of partial nitrification. Therefore, its actual concentration in the effluent can be considered a suitable parameter for evaluating the stability of partial nitrification. Simultaneously, the ratio of  $\text{NO}_2^-$ -N to  $\text{NH}_4^+$ -N concentration can be used for this purpose. The alkalinity of used reject water enables the conversion of ca. 50–60% of  $\text{NH}_4^+$ -N contained in the reject water to  $\text{NO}_2^-$ -N without pH control so long as the process is not limited by other factors [9]. Considering the fact that  $\text{NH}_4^+$ -N was converted primarily to  $\text{NO}_2^-$ -N without intensive  $\text{NO}_3^-$ -N production, a significant decrease of the  $\text{NO}_2^-$ -N: $\text{NH}_4^+$ -N ratio below 1.0 may indicate a decrease in AOB activity. Under these conditions, the disruption of partial nitrification stability may be signalled by a decrease in the  $\text{NO}_2^-$ -N: $\text{NH}_4^+$ -N ratio.

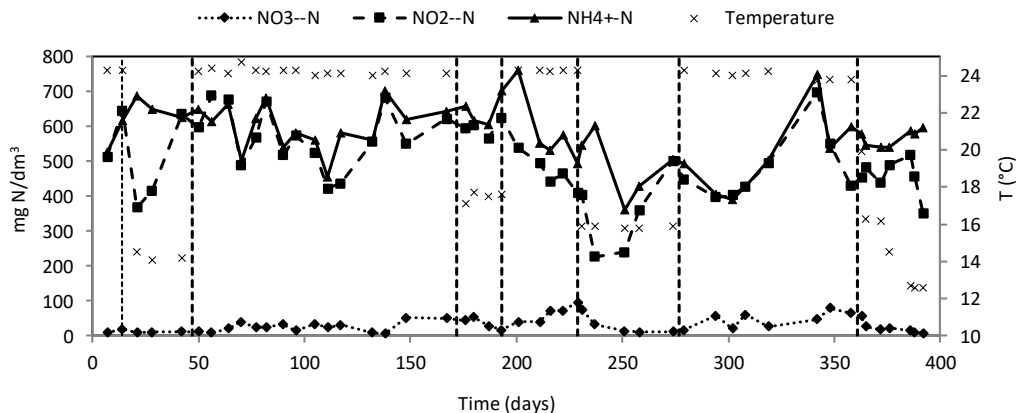


Fig. 2. The concentrations of different nitrogen species in the effluent during operational periods 1–8

$\text{NO}_2^-$ -N concentration ranged between 230 and 680  $\text{mg}/\text{dm}^3$  throughout the entire reactor operation (Fig. 2). The lowest concentrations were recorded after a temperature decrease at operational periods 2 and 6, indicating instability of the treatment process. Consequently, the amount of  $\text{NO}_2^-$ -N in the reactor effluent gradually increased during these periods, indicating gradual re-activation of AOBs. Simultaneously, the average ratio of  $\text{NO}_2^-$ -N to  $\text{NH}_4^+$ -N concentration was lower during operational periods 2 (0.56) and 6 (0.67) compared with periods 1 (1.0), 3 (0.95), 5 (0.81) and 7 (0.95). No significant changes in concentration of nitrogen species were recorded during period 8 until day 388. However, a decrease in  $\text{NO}_2^-$ -N from 520 to 352  $\text{mg}/\text{dm}^3$  (Fig. 2) and simultaneous fall of the ratio of  $\text{NO}_2^-$ -N to  $\text{NH}_4^+$ -N concentration from 0.92 to 0.59 was observed after the decrease in temperature to 12.7 °C between days 386 and 392.

Unexpectedly, the above mentioned decreases in  $\text{NO}_2^-$ -N concentration after temperature falls (periods 2, 6 and end of period 8) were not combined with an obvious simultaneous increase in  $\text{NH}_4^+$ -N concentration (Fig. 2). The representation of volatile FA within  $\text{NH}_4^+$ -N increases rapidly upon increasing pH [18]. In accordance with this statement, FA concentration increased significantly during period 2, 6 and 8 (Figs. 1 and 3) and it is necessary to consider the possibility of intensive stripping of FA during these periods in an intensively aerated system. This hypothesis was confirmed by the decrease in nitrogen concentration during these phases. For example, during day 251 (period 6), total inorganic nitrogen concentration in the effluent (the sum of  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N) reached 620  $\text{mg}/\text{dm}^3$  at an  $\text{NH}_4^+$ -N input concentration of 1020  $\text{mg}/\text{dm}^3$  indicating that almost 40% of the nitrogen was stripped out. Simultaneously, no significant nitrogen loss was observed during the stable partial nitrification (e.g. periods 1, 3 and 5). Considering the fact that ammonia was removed not only by partial nitrification but also by an additional mechanism during the selected periods of reactor operation,  $\text{NH}_4^+$ -N



removal efficiency seems not to be sufficiently objective for the purpose of comparing partial nitrification stability during individual periods of reactor operation.

*FA and FNA concentrations and their effect on the treatment process.* Development of FA and FNA concentrations during the experiment is recorded in Figs. 3 and 4.

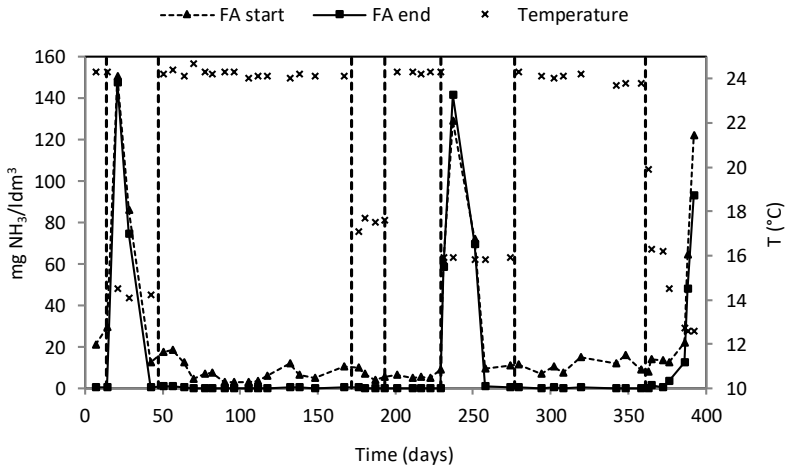


Fig. 3. Concentration of FA at the beginning and at the end of the SBR cycle

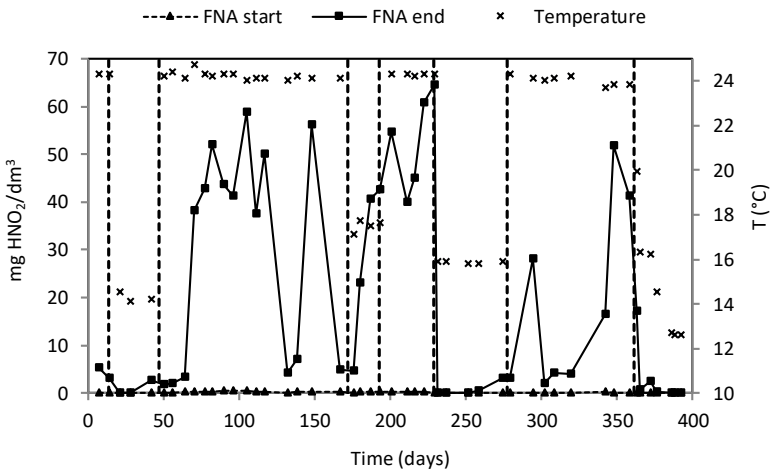


Fig. 4. Concentration of FNA at the beginning and at the end of the SBR cycle

FA concentration reached its maximum at the beginning of the SBR cycle (3–20 mg NH<sub>3</sub>/dm<sup>3</sup>), while FNA concentration increased during the cycle, reaching its maximum at the end of the cycle (up to 61 mg HNO<sub>2</sub>/dm<sup>3</sup>) in the case of stable operation of partial nitrification in accordance with [17]. NOB activity was suppressed due to FA and/or FNA

inhibition, while AOB activity was preserved under these conditions thanks to their higher resistance [18]. An interesting observation is that the activity of AOB was also sufficient for successful partial nitrification at extremely high values of FNA at the end of the cycle in periods 3, 5 and 7 (Fig. 4). This could be caused by the adaptation of the microorganisms to the prevailing conditions within the reactor [1]. On the other hand, the finding that only ca. 50% of  $\text{NH}_4^+\text{-N}$  was removed by AOB during periods with stable partial nitrification (periods 1, 3, 4, 5, 7) indicates that AOB were in some way inhibited at a certain FNA concentration with pH gradually decreasing and FNA concentration gradually increasing during the SBR cycle. Consequently, they were probably reactivated at the beginning of the subsequent cycle in connection with a pH increase and simultaneous FNA concentration decrease. This hypothesis is in accordance with the results of literature source [28] evaluating the effect of pH and FNA on the activity of AOB in a partial nitrification reactor. Significant increases in pH throughout the whole cycle of the SBR recorded during selected phases of periods 2, 6 and 8 (Fig. 1) resulted in an extreme increase in FA concentration. For example, as much as  $150 \text{ mg/dm}^3$  FA was measured on day 21 (period 2, Fig. 3). Therefore, AOB activity limited primarily by a decrease in temperature was also inhibited secondarily by FA [18]. This probably prolonged the period required for the recovery of satisfactory AOB activity during phases 2 and 6. A significant increase in FA concentration (up to  $122 \text{ mg NH}_3/\text{dm}^3$  at the beginning of the cycle) was also detected after the decrease in temperature to  $12.7 \text{ }^\circ\text{C}$  realised within period 8, resulting in a similar secondary inhibition effect.

*Representation of  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N within oxidized forms of nitrogen.*  $\text{NO}_2^-$ -N represented 86–98% of oxidized nitrogen (the sum of  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N) in the effluent from the reactor for the whole reactor operation. This suggests effective and long-term restriction of NOB activity. The literature generally indicates the accumulation of nitrites is supported by higher temperatures [29]. However, even the temperature decreases

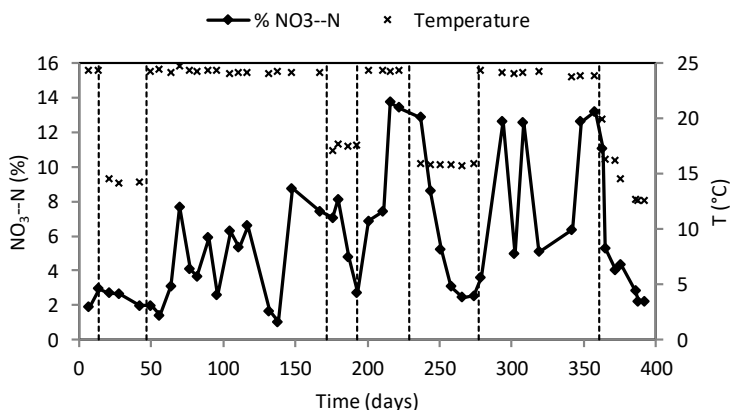


Fig. 5. Representation of  $\text{NO}_3^-$ -N between oxidized forms of nitrogen

realized within periods 2, 4, 6 and 8 did not induce a significant increase in  $\text{NO}_3^-$ -N effluent concentration within this study. To the contrary, the representation of  $\text{NO}_3^-$ -N between nitrogen oxidized forms decreased with decreasing temperatures (Fig. 5). Simultaneously, a certain increase in the representation of  $\text{NO}_3^-$ -N within oxidized nitrogen, indicating an undesirable increase of NOB activity, was repeatedly recorded after temperature increases (periods 3, 5, 7) compared to previous periods with decreased temperature (periods 2, 4, 6). For example, the representation of  $\text{NO}_3^-$ -N increased from 3 to 14% during period 5 (Fig. 5). The strong inhibition of NOB activity (due to extreme FA concentration increases during periods 2, 6 and 8 and consequently decreases during periods 3, 4, 5 and 7) could be the main factor causing this fact. Also, the faster growth rate of NOB under higher temperatures [30] could support this phenomenon. In all cases, the representation of  $\text{NO}_3^-$ -N within oxidized forms of nitrogen did not exceed 14% during the whole reactor operation time and the fluctuation of  $\text{NO}_3^-$ -N representation had no fatal effect on the process performance.

#### 4. CONCLUSIONS

It was found that a sudden decrease in temperature from 24.3 °C to 17.4 °C did not affect the stability of partial nitritation used for reject water treatment under the conditions applied within the described experiment, indicating relatively good temperature stability within the tested system. In addition, during a gradual temperature decrease, the system was stable until 12.7 °C. Based on these findings, the operation of full-scale reactors treating reject water without temperature control under the conditions described in this study seems to be achievable. Quick decreases in temperature from 24.3 °C to 14.3 and 15.8 °C caused significant disruptions to process stability indicating that too intense and sudden decreases in temperature may deteriorate the performance of the process on a relatively long-term basis, even at a temperature higher than the value acceptable in the case of its gradual decrease. The secondary inhibition effect induced by elevated concentrations of FA may support the primary restriction of AOB activity caused by a decrease in temperature with the potential to significantly prolong the phase of deteriorated function of the system. However, the ability of AOB to adapt to low temperature values was proven by gradual restoration of the stability of partial nitritation even after sudden a decrease in temperature from 24.3 to 14.3 °C. Therefore, long term operation at a lowered temperature seems to be achievable. However, the ability of AOB to adapt to low temperature were proved by gradual restoration of the stability of partial nitritation even after sudden decrease of temperature from 24.3 to 14.3 °C. Therefore, long term operation at lowered temperature seems to be achievable. The results of this paper are fully applicable for the reactors applying partial nitritation as the first stage before independent anammox reactor. Simultaneously, basic findings could

be used for the control of reactors applying full nitrification within the system for nitrification/denitrification operated in two separated reactors. For objective evaluation of temperature stability of one-step systems of nitrification/denitrification and partial nitrification/anammox additional research is needed.

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#### REFERENCES

- [1] TURK O., MAVINIC D.S., *Stability of nitrite build-up in an activated sludge system*, J. WPCF, 1989, 61 (8), 1440.
- [2] ABELLING U., SEYFRIED C.F., *Anaerobic-aerobic treatment of high-strength ammonium wastewater-nitrogen removal via nitrite*, Water Sci. Technol., 1992, 26 (5/6), 1007.
- [3] MULDER A., VAN DE GRAAF A.A., ROBERTSON L.A., KUENEN J.G., *Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor*, FEMS Microbiol. Ecol., 1995, 16 (3), 177.
- [4] JETTEN M.S.M., WAGNER M., FUERST J., VAN LOOSDRECHT M., KUENEN G., STROUS M., *Microbiology and application of the anaerobic ammonium oxidation (anammox) process*, Curr. Opin. Biotech., 2001, 12 (3), 283.
- [5] KARTAL B., KUENEN J.G., VAN LOOSDRECHT M.C.M., *Sewage treatment with anammox*, Science, 2010, 328 (5979), 702.
- [6] KARTAL B., DE ALMEIDA N.M., MAALCKE W.J., DEN CAMP H.J.M.O., JETTEN M.S.M., KELTIJENS J.T., *How to make a living from anaerobic ammonium oxidation*, FEMS Microbiol. Rev., 2013, 37 (3), 428.
- [7] STROUS M., HEIJNEN J.J., KUENEN J.G., JETTEN M.S.M., *The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms*, Appl. Microbiol. Biot., 1998, 50, (5), 589.
- [8] HELLINGA C., SCHELLEN A.A.J.C., MULDER J.W., VAN LOOSDRECHT M.C.M., HEIJNEN J.J., *The SHARON process. An innovative method for nitrogen removal from ammonium-rich waste water*, Water Sci. Technol., 1998, 37 (9), 135.
- [9] JENICEK P., SVEHLA P., ZABRANSKA J., DOHANYOS M., *Factors affecting nitrogen removal by nitrification/denitrification*, Water Sci. Technol., 2004, 49 (5/6), 73.
- [10] LACKNER S., GILBERT E.M., VLAEMINCK S.E., JOSS A., HORN H., VAN LOOSDRECHT M.C.M., *Full-scale partial nitrification/anammox experiences. An application survey*, Water Res., 2014, 55, 292.
- [11] SLIEKERS A.O., DERWORT N., GOMEZ J.L.C., STROUS M., KUENEN J.G., JETTEN M.S.M., *Completely autotrophic nitrogen removal over nitrite in one single reactor*, Water Res., 2002, 36, 2475.
- [12] VAN DONGEN U., JETTEN M.S.M., VAN LOOSDRECHT M.C.M., *The SHARON<sup>®</sup>-Anammox<sup>®</sup> process for treatment of ammonium rich wastewater*, Water Sci. Technol., 2001, 44 (1), 153.
- [13] VAN KEMPEN R., MULDER J.W., UIJTERLINDE C.A., LOOSDRECHT M.C.M., *Overview. Full scale experience of the SHARON<sup>®</sup> process for treatment of rejection water of digested sludge dewatering*, Water Sci. Technol., 2001, 44 (1), 145.
- [14] RUIZ G., JEISON D., CHAMY R., *Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration*, Water Res., 2003, 37 (6), 1371.
- [15] JIANLONG W., NING Y., *Partial nitrification under limited dissolved oxygen conditions*, Proc. Biochem., 2004, 39 (10), 1223.

- [16] POLLICE A., TANDOI V., LESTINGI C., *Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate*, Water Res., 2002, 36 (10), 2541.
- [17] SVEHLA P., BARTACEK J., PACEK L., HRNCIROVA H., RADECHOVSKY J., HANC A., JENICEK P., *Inhibition effect of free ammonia and free nitrous acid on nitrite-oxidising bacteria during sludge liquor treatment. Influence of feeding strategy*, Chem. Pap., 2014, 68 (7), 871.
- [18] ANTHONISEN A.C., LOEHR R.C., PRAKASAM T.B.S., SRINATH E.G., *Inhibition of nitrification by ammonia and nitrous acid*, J. Water Poll. Contr. Fed., 1976, 48 (5), 835.
- [19] BLACKBURNE R., YUAN Z., KELLER J., *Demonstration of nitrogen removal via nitrite in a sequencing batch reactor treating domestic wastewater*, Water Res., 2008, 42 (8/9), 2166.
- [20] PAMBRUN V., PAUL L., SPÉRANDIO M., *Control and modelling of partial nitrification of effluents with high ammonia concentrations in sequencing batch reactor*, Chem. Eng. Proc., 2008, 47 (3), 323.
- [21] HENZE M., VAN LOOSDRECHT M.C.M., EKAMA G., BRDJANOVIC D., *Biological wastewater treatment: principles, modelling and design*, IWA Publishing, London 2008.
- [22] KIM J.-H., GUO X., PARK H.-S., *Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation*, Process Biochem., 2008, 43 (2), 154.
- [23] VOLCKE E.I.P., LOCCUFIER M., VANROLLEGHEM P.A., NOLDUS E.J.L., *Existence, uniqueness and stability of the equilibrium points of a SHARON bioreactor model*, J. Process Control, 2006, 16 (10), 1003.
- [24] RODRÍGUEZ D.C., PINO N., PEÑUELA G., *Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a sequencing batch reactor (SBR)*, Biores. Technol., 2011, 102 (3), 2316.
- [25] LÓPEZ-PALAU S., SANCHO I., PINTO A., DOSTA J., MATA-ÁLVAREZ J., *Influence of temperature on the partial nitrification of reject water in a granular sequencing batch reactor*, Environ. Technol., 2013, 34 (18), 2625.
- [26] APHA, *Standard Methods for examination of Water and wastewater*, 19th Ed., American Publishers Health Association, Washington DC, USA, 1995.
- [27] PARK S., BAE W., *Modelling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid*, Proc. Biochem., 2009, 44 (6), 631.
- [28] CLAROS J., JIMÉNEZ E., AGUADO D., FERRER J., SECO A., SERRALTA J., *Effect of pH and HNO<sub>2</sub> concentration on the activity of ammonia-oxidizing bacteria in a partial nitrification reactor*, Water Sci. Technol., 2013, 67 (11), 2587.
- [29] FORD D.L., CHURCHWELL R.L., KACHTICK J.W., *Comprehensive analysis of nitrification of chemical processing wastewater*, J. Water Poll. Contr. Fed., 1980, 52 (11), 2726.
- [30] PAINTER H., *A review of literature on inorganic nitrogen metabolism in microorganisms*, Water Res., 1970, 4, 395.