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Direct and indirect effects of oxygen limitation on nitrification process applied to reject water treatment

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ABSTRACT

The direct and indirect influence of temporary oxygen limitation on the nitrification of reject water was investigated using a continuous stirred tank bioreactor operated for 330 d at laboratory temperature (23 ± 1°C). A decrease in dissolved oxygen (DO) concentration from 3 to 0.7 mg L^{-1} lasted for 38 d and led to effective nitrite accumulation—more than 95% of total oxidized nitrogen was present as nitrite. The drop of DO concentration, at the same time, caused a decrease in the nitrogen oxidation rate from 1.36 to 0.73 kg N m^{-3} d^{-1}. After a subsequent DO concentration increase to 3 mg L^{-1}, the nitrite accumulation remained stable for another 90 d. This development was caused mainly by the indirect effects of DO limitation, consisting especially in the change of nitrogen species represented. A significant increase in free nitrous acid concentration induced by temporary DO limitation seems to be the key factor in this respect. The results of Fluorescence *in situ* hybridization analysis confirmed a washout of nitrite-oxidizing bacteria (NOB) during the period with high nitrite accumulation. The representation of NOB in total biomass decreased from 6.4% to less than 1% as a consequence of temporary DO limitation.

Keywords: Ammonia-oxidizing bacteria; Free ammonia; Free nitrous acid; Nitrite-oxidizing bacteria; Oxygen limitation; Partial nitrification; Reject water

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1. Introduction

The separate treatment of water rejected from anaerobic digestion of sewage sludge represents the possibility to achieve highly efficient nitrogen removal with a low cost per volume unit of wastewater [1]. Nitrogen compounds in the form of ammonium nitrogen (expressed as total ammonium nitrogen— [TAN]) are commonly eliminated from wastewater by a combination of two processes: nitrification and denitrification. The specific composition of reject water (high

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TAN concentration, low chemical oxygen demand/nitrogen— [COD/N]— ratio) enables new alternatives in the biological nitrogen removal processes based on partial nitrification, converting TAN to nitrite (NO_2^-) instead of nitrate (NO_3^-), followed by subsequent nitrogen reduction via denitritation (denitrification of N-NO_2^-) [2] or anaerobic ammonium oxidation (ANAMMOX technology) [3,4]. Benefits of both methods were thoroughly described in the literature [5] and are generally known.

Partial nitrification is based on the limitation of nitrite-oxidizing bacteria (NOB) activity while maintaining the activity of ammonia-oxidizing bacteria (AOB). AOB and NOB prefer different pH values [6] and temperature [7], and they are not equally sensitive to low dissolved oxygen (DO) concentrations [8,9], changes of oxic conditions form anaerobic to aerobic [10,11], low sludge retention time (SRT) [7], toxic nitrogen compounds (free ammonia—[FA] and free nitrous acid—[FNA]) [12–15] or elevated HCO_3^- concentrations [16]. Through the different selective factors, it is possible to keep AOB active, while the growth of NOB is suppressed and its activity restricted.

The method of DO control may be considered as one of the most reliable and relatively "low-cost" NOB washout strategies. The exact conventional Monod-type half-saturation constant for DO concentration (K_{DO}) differs according to the conditions applied in given experiments described in the literature (Table 1), but it is generally accepted that the saturation constant for AOB is lower than that for NOB [8], that is, if the DO value is set higher than K_{DO} for AOB, but still lower than K_{DO} for NOB, partial nitrification can occur under certain conditions [8,17,20]. Oxygen is used preferentially for TAN oxidation, which could disadvantage NOB in the case of DO insufficiency. N-NO_2^- accumulation in continuous stirred systems with biomass in suspension, treating wastewater with high TAN concentration, was usually observed at concentrations below 1.5 mg L^{-1} [17,20–23]. Blackburne et al. [17] mentioned a concentration of 0.4 mg L^{-1} to be sufficient for an efficient partial nitrification. Ruiz et al. [20] reported the optimal DO concentration for achieving partial nitrification together with high conversion efficiency was 0.7 mg L^{-1}, but a concentration lower than 0.5 mg L^{-1} had an adverse effect on TAN oxidation efficiency, while Wang and Yang [21] found the optimal DO value for N-NO_2^- accumulation at 1.5 mg L^{-1}. Jubany et al. [22] applied partial nitrification within a range between 1.2 and 1.9 mg L^{-1}, while Wang et al. [23] achieved the best performance of a nitritation reactor treating landfill leachate at DO concentrations between 1 and 2 mg L^{-1}. The effects of DO control could be effectively combined with other factors inhibiting NOB activity (lowered HRT, toxic effect of FA, etc.) in order to achieve efficient nitrite accumulation, even during the nitrification process of wastewater with a TAN concentration lower than 100 mg L^{-1} [24].

Table 1
K_{DO} according to different authors

Reference	AOB (mg L^{-1})	NOB (mg L^{-1})
[8]	0.74	1.75
[17]	0.03	0.4
[18]	0.3	1.1
[19]	0.5	1.9

The direct effects of oxygen limitation on NOB activity has been thoroughly studied recently and is currently described well. The effective partial nitrification of TAN-concentrated water achieved through DO control has been reported by many authors [6,9,17,20–23]. Concurrently, the secondary effects of oxygen limitation consisting in the lag phase of NOB activity after switching the anoxic/anaerobic conditions to aerobic conditions has been discussed in the past [25]. As a result of these findings, many authors applied a strategy based on intermittent aeration, where the crucial principle is to operate the system at an optimal length of aerobic phases in the reactors, combining the anoxic and aerobic processes. Most frequently, wastewater with a relatively low TAN concentration has been treated on this principle in SBR reactors [9,10]. However, additional indirect effects of oxygen limitation could be expected in the case that wastewater with an extremely high TAN concentration (such as reject water) is treated in a fully aerobically operated nitrification reactor without anoxic/anaerobic phases. The change of the N-NO_3^-/N-NO_2^- ratio and, alternatively, the decrease of TAN removal efficiency caused by limited DO availability [20] lead to the increase of FNA and FA concentration in the reactor [26]. Therefore, the simultaneous inhibition of NOB caused by a limited DO concentration together with high FNA and, potentially, FA should be expected when treating reject water if DO limitation is applied as the main primary factor used for the selection of nitrifying bacteria. This aspect of DO limitation has not been seriously evaluated yet.

Within this paper, DO limitation during separate reject water treatment realized in continuously stirred tank reactor (CSTR) with flocculent biomass was applied as a primary factor inhibiting NOB activity in a lab scale. The main objective of this study was to evaluate the indirect effect of DO limitation on nitrite and nitrate accumulation in the reactor and long-term restriction of NOB activity.

2. Experimental

The experiment was performed at Czech University of Life Sciences Prague, Department of Agro-Environmental Chemistry and Plant Nutrition from September 17, 2011 to July 28, 2012. The Fluorescence *in situ* hybridization (FISH) analyses were conducted at the Institute of Chemical Technology in Prague, Department of Water Technology and Environmental Engineering.

2.1. Influent

Raw reject water obtained from the Prague Central Wastewater Treatment Plant was used as the influent substrate for the experiment. The chemical characteristics are shown in Table 2.

2.2. Reactor setup

The model reactor was designed as a CSTR with an attached settler, enabling sludge recirculation (Fig. 1). The operating reactor volume was 1.5 L. Activated sludge from another experimental reactor treating reject water to the final nitrification product N-NO_3^- was used as the inoculum. The reactor was filled with 0.5 L of the sludge (volatile suspended solids [VSS] 5.35 g L^{-1}) and 1 L of tap water. The influent was fed into the reactor by a peristaltic pump (Kouril PCD 21, Czech Republic). Reactor mixing as well as basic coarse bubble aeration was provided continuously by a small aquarium air pump (Tetratec APS 400, Germany). Based on similar conditions applied during the experiment described by Ruiz et al. [20], a DO concentration of 0.7 mg L^{-1} was applied in order to initiate partial nitrification. The DO concentration was kept within the adjusted interval by the intermittent aeration of an additional aquarium air pump controlled by a computer with an attached DO sensor. In order to achieve maximum nitrogen oxidation efficiency (NOE), it was necessary to compensate HCO_3^- deficiency, or more precisely, the insufficient ratio between inorganic carbon and TAN [27,28] followed by a pH decline induced by AOB activity via the dosing of an NaOH solution. In this way, the pH value was set to 7 (with a hysteresis of 0.05). The experiment was performed at laboratory temperature (23 ± 1℃). SRT was not purposely limited, and no excess sludge was taken from the reactor, excluding the biomass lost from the reactor with the effluent from secondary sedimentation. Thanks to this fact, very long SRT (at least 25 d) was applied during the experiment. The actual SRT value could not be exactly measured due to a high and variable concentration of VSS and total suspended solids (TSS) in the influent.

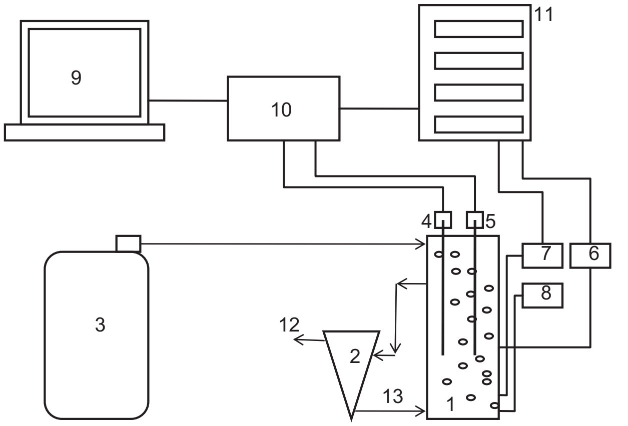

Fig. 1. Schematic diagram of a setup with attached DO and pH control unit.
Notes: (1) nitrification CSTR reactor, (2) settler, (3) reject water tank, (4) pH electrode, (5) DO electrode, (6) pH regulation pump, (7) aeration, (8) reactor mixing via air bubbles, (9) PC, (10) interface, (11) automatic switches controlled by PC, (12) effluent, and (13) sludge recirculation.

Table 2
Chemical characteristics of the influent reject water. Chemical oxygen demand of total solids (COD_{TS}); chemical oxygen demand of dissolved solids (COD_{DS})

	Average
pH	8.42 ± 0.22
TAN (mg L^{-1})	1.450 ± 165
P-PO_4^{3-} (mg L^{-1})	34 ± 8
Alkalinity (mmol L^{-1})	97 ± 6.3
COD_{TS} (mg L^{-1})	2.675 ± 770
COD_{DS} (mg L^{-1})	1.665 ± 510
TSS (g L^{-1})	1.14 ± 0.50
VSS (g L^{-1})	1.00 ± 0.48

2.3. Operational phases

The operation period of the reactor was divided into four phases (Table 3).

The NLR value was intentionally regulated only during Phase I by a gradual increase of the flow rate. During Phases II–IV, the flow rate was kept stable, so the fluctuations of NLR were caused only by variable TAN concentrations in the influent reject water. Average values of NLR were comparable for Phases II–IV.

Table 3
Reactor conditions and operation length of the experimental phases

Phase number	Operational period (d)	Average NLR* (kg TAN m^{-3} d^{-1})	Set DO conc. (mg L^{-1})
I	1–105	0.13 – 1.50**	3.0
II	106–142	1.42 ± 0.06	3.0
III	143–182	1.32 ± 0.12	0.7
IV	183–330	1.13 ± 0.24	3.0

*NLR—nitrogen loading rate

**NLR gradually increased from 0.13 to 1.50 kg TAN/(m^3 d).

The start-up phase (Phase I) lasted for 105 d with the aim to achieve an NLR value approaching 1.65 kg N m^{-3} d^{-1}, the maximum achievable under given conditions [27]. Phase II lasted for 36 d with the aim to stabilize the system operated under high NLR with unlimited DO. Phase III lasted for 39 d in order to operate the system under limited DO for a period of time comparable with Phase II. Phase IV lasted for 147 d with the aim to evaluate the characteristics of the system after the termination of DO limitation.

2.4. Analytical methods

The following basic physical and chemical analyses were performed during the experiment: pH value, DO concentration, temperature, and the concentration of TAN, and N-NO_2^- and N-NO_3^-, COD, TSS, and VSS.

The pH value, DO concentration, and temperature were monitored continuously online (Gryf Magic XBC device, Gryf HB company, Czech Republic). The pH value was measured by an ISE electrode PCL 321 XB2, and DO was measured by a membrane electrode KCL 24 XB4. Both types of electrodes were equipped with temperature sensors. The concentration of observed nitrogen compounds, COD_{TS}, and COD_{DS} was measured weekly using a HACH DR/4000 spectrophotometer (HACH LANGE GmbH). The analysis of TAN, N-NO_2^-, and COD was performed in accordance with standard methods [29], and N-NO_3^- was analyzed according to the HACH 2511 method with the application of HACH test kits purchased at HACH LANGE GmbH. The samples were taken from the input reject water and from the settler once a week. Alkalinity was determined by titration of the sample with hydrochloric acid (0.1 mol L^{-1}) up to pH 4.5.

Analyses of VSS were performed every week according to the standard methods [29] in order to quantify the activated sludge biomass. The samples were taken from the nitrification CSTR reactor, where the homogeneity of suspension was guaranteed by a continuous aeration of the reactor (see above).

2.5. Microbial analyses

In order to reveal the microbial structure of the nitrifying biomass, the FISH analysis was performed. AOB communities were examined with the NSO mix (NSO190 + NSO1225) probe, detecting (β-proteobacterial AOB), dye Fluos, stained green in color. NOB were quantified with the Ntspa mix (Ntspa712+Ntspa662) probe, detecting whole phylum *Nitrospirae* species, and NIT3, detecting *Nitrobacter* species, dye Cy3, stained red in color. FISH images were collected using an Olympus BX51-RFAA epifluorescence microscope with a charge-coupled device camera. FISH quantification was performed according to Daims et al. [30], where the relative abundance of each group was determined in triplicate as mean percentage of the target bacteria. The result determines the percentage expression of the area occupied by nitrifying bacteria from the area of total biomass.

2.6. Calculations

The following formulas were used to quantify indicators describing the process conditions.

Nitrogen oxidation efficiency (NOE, %)

$$\mathrm{NOE} = \frac{C_{\mathrm{N-NO_2^-}} + C_{\mathrm{N-NO_3^-}}}{C_{\mathrm{TAN-in}}} \cdot 100\% \tag{1}$$

$C_{\text{TAN-in}}$ (mg L^{-1})—TAN concentration measured in the influent.

$C_{\mathrm{N-NO_2^-}}$ (mg L^{-1})—N-NO_2^- concentration measured in the effluent.

$C_{\mathrm{N-NO_3^-}}$ (mg L^{-1})—N-NO_3^- concentration measured in the effluent.

Nitrogen oxidation rate (NOR, kg TAN m^{-3} d^{-1}).

$$\mathrm{NOR} = \frac{\mathrm{NLR} \cdot \mathrm{NOE}}{100} \tag{2}$$

NLR (kg TAN m^{-3} d^{-1})—nitrogen loading rate.

Specific nitrogen oxidation rate ([sNOR], mg N g VSS^{-1} d^{-1}).

$$\mathrm{sNOR} = \frac{\mathrm{NOR}}{\mathrm{VSS}} \cdot 1{,}000 \tag{3}$$

VSS (g/L)—concentration of activated sludge expressed as VSS.

FA and FNA nitrogen were calculated according to Anthonisen et al. [12] and modified in order to express only the concentration of nitrogen occurring in the form of FA or FNA ($C_{\mathrm{N-FA}}$, $C_{\mathrm{N-FNA}}$, mg L^{-1}).

$$C_{\mathrm{N-FA}} = \frac{C_{\mathrm{TAN}} \cdot 10^{\mathrm{pH}}}{\exp(6{,}334/(273+t)) + 10^{\mathrm{pH}}} \tag{4}$$

C_{TAN}(mg L^{-1})—TAN concentration measured in the reactor.

t (℃)—temperature.

$$C_{\mathrm{N-FNA}} = \frac{C_{\mathrm{N-NO_2^-}}}{[\exp(-2{,}300/(273+t)) + 10^{\mathrm{pH}}] + 1} \tag{5}$$

$C_{\mathrm{N-NO_2^-}}$(mg L^{-1})—N-NO_2^- concentration measured in the reactor.

t (℃)—temperature.

2.7. Statistical analyses

Data and statistical analyses were calculated using STATISTICA 12.0 software (StatSoft, Tulsa, USA) and MS Excel 2010. The homogeneity of group variances were checked using the *F*-test, and NOE and NOR values were examined by Kruskal–Wallis' one-way analysis of variance. The level of significance was set at $\alpha < 0.05$.

3. Results and discussion

3.1. Fluctuation of DO concentration

DO concentration during Phase I, II, and Phase IV was kept at an average level of 2.85 ± 0.48 mg L^{-1}, with 90% of continuously recorded values within the interval of 2.3–3.8 mg L^{-1}. Typical DO oscillation during Phase I, II, and Phase IV is shown in Fig. 2(A). The decrease of DO concentration caused by nitrification activity was always followed by an increase of DO concentration when the automatic switch turned on additional aeration.

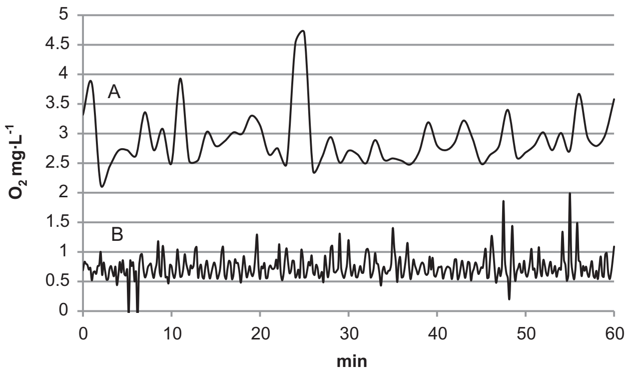

Fig. 2. Typical DO level oscillation during Phases I, II, and IV (**A**) and Phase III (B).

Phase III was initiated on day 146 with the adjusted decline of DO concentration from 3.0 to 0.7 mg L^{-1}. Fig. 2(B) covers a 1-h period of DO oscillation during Phase III. The changes in DO concentration were considerably faster during Phase III compared to Phases I, II, and IV. This development was caused by the narrower hysteresis interval together with the rapid change of DO concentration when the aeration was set on. Although maintaining a stable DO concentration in the oxygen limited conditions of the reactor with the volume of 1.5 L by conventional bubble aeration was challenging, the average value was 0.73 ± 0.20 mg L^{-1}.

3.2. Phases I and II—nitrification activity without DO limitation

During Phase I, NLR was gradually increased from 0.13 to 1.5 kg TAN m^{-3} d^{-1} from day 1 to 105 (Fig. 3(A)). NOE fluctuated between 94.4 and 99.8%, except for a short, initial 30-d period (Fig. 3(A)). N-NO_3^- was the main product of nitrification starting on day 24, and the N-NO_2^-/N-NO_x^- concentration ratio did not exceed 1% (except the short aeration failure around the day 90—Fig. 3(B)).

Phase II was characterized by stable NLR (average 1.42 ± 0.06 kg TAN m^{-3} d^{-1}) and a DO concentration within the same range as in Phase I. NOR at the highest NLR reached 1.47 kg TAN m^{-3} d^{-1}. The average sNOR reached 192.6 ± 8.5 mg N g VSS^{-1} d^{-1}. Similarly as in Phase I, N-NO_3^- was the main nitrification product during Phase II (Fig. 3(B)).

3.3. Phase III—direct effect of DO limitation

The low DO concentration of 0.7 mg L^{-1} maintained during Phase III was close to the half-saturation constant of AOB reported by Guisasola et al. [8]. The ratio between oxidized nitrogen forms switched

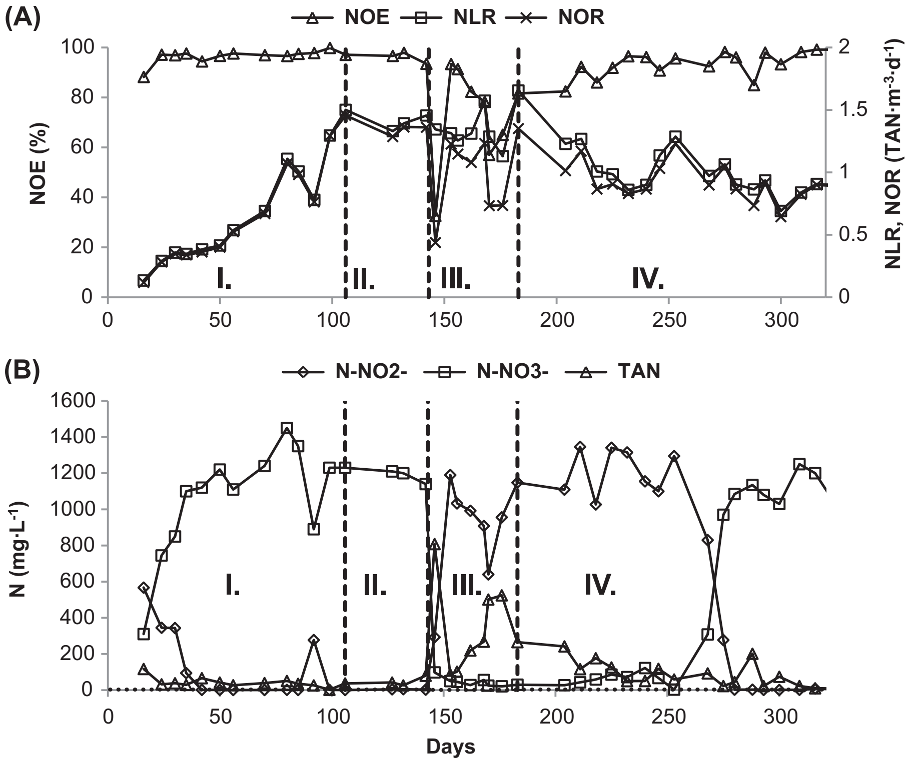

Fig. 3. (A)—NOE, NLR, and NOR during the whole reactor operation period. (B)—Concentration of TAN and oxidized nitrogen compounds during the whole operation period. Vertical dashed lines in figures (A) and (B) indicate transitions between phases with various DO concentrations.

completely within 10 d after the decrease of DO concentration. While the activity of AOB remained relatively high (sNOR 167 mg $\mathrm{N\,g\,VSS^{-1}\,d^{-1}}$), NOB activity declined significantly, inducing an increase of the $\mathrm{N\text{-}NO_2^-/N\text{-}NO_x^-}$ concentration ratio to 94.2–97.9% during all of Phase III (Fig. 4(A)).

However, the NOB suppression, together with the decrease in DO, resulted in NOE instability. NOE fluctuated between 65.1 and 93.4% during Phase III, when a significantly lower value (32.6%) was registered within the first measurement performed on day 146 (Fig. 3(A)). These results are in agreement with the observation of Wang et al. [23], who mentioned significant NOE decline with DO below 1.0 $\mathrm{mg\,L^{-1}}$ during the treatment of concentrated landfill leachates. The opposite development was observed by Ruiz et al. [20]. Under comparable DO and NLR conditions and a higher temperature of 30°C, the authors did not detect any decrease of NOE as a consequence of DO limitation.

3.4. Phase IV—indirect effect of DO limitation

Phase IV started on day 183 with an increase of DO concentration up to the original 3 $\mathrm{mg\,L^{-1}}$. As a result, NOE increased to the average value of 93.5 ± 5.5% during this phase (Fig. 3(A)).

The termination of DO limitation did not result in increased NOB activity followed by an increase of $\mathrm{N\text{-}NO_3^-}$ concentration in the reactor. On the contrary, almost all oxidized nitrogen was present as $\mathrm{N\text{-}NO_2^-}$ during the subsequent 90 d (Fig. 4(A)), while the $\mathrm{N\text{-}NO_2^-/N\text{-}NO_x^-}$ concentration ratio did not fall below 90%. Such observations contradict the studies of Ciudad et al. [6], Bae et al. [31], Peng et al. [32], and Wang et al. [23] who all reported that high DO decreases or completely disrupts the stable process of $\mathrm{N\text{-}NO_2^-}$ accumulation in TAN-concentrated wastewater, even after a long-term operation of several days or up to weeks at a maximum. The effective washout of NOB during Phase III and the consequent very long period of their recovery of activity are indicated by these findings. Certain indirect effects of oxygen limitation seem to be responsible for a significant extension of the period with effective nitrite accumulation observed in Phase IV. This phenomenon consists especially in the change of nitrogen species represented in the reactor, induced primarily by DO limitation in Phase III.

An increase of $\mathrm{N\text{-}NO_2^-}$ concentration in the reactor from several units of $\mathrm{mg\,L^{-1}}$ at a maximum during

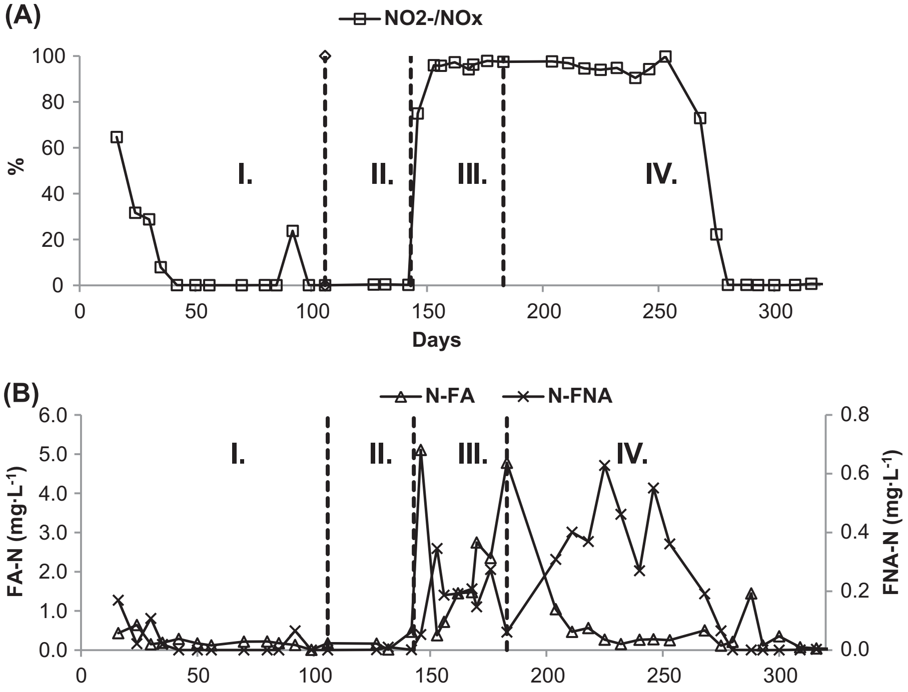

Fig. 4. (A)—N-NO_2^-/N-NO_x performance during the whole operation period. (B)—Concentration of FA and FNA during the whole operation period. Vertical dashed line indicates transition between phases with various DO concentrations.

Phase II to more than a thousand mg L^{-1} registered during Phase III, as well as a substantial part of Phase IV (Fig. 3(B)), resulted in a significant raise of FNA concentration, which formed 0.06–0.63 mg N L^{-1} between days 153 and 268 (Fig. 4(B)). This situation very probably prolonged the period of the recovery of NOB activity, because the concentration of FNA causing NOB inhibition presented in the literature ranges between 0.02 and 0.45 mg N L^{-1} [12–14,22,33]. Furthermore, even dissociated N-NO_2^- itself, present in extremely high concentrations in the reactor during the initial 90 d of Phase IV (Fig. 3(A)), was mentioned as an NOB inhibitor [34]. Simultaneously, the DO limitation caused an NOE decline during Phase III (see above) resulting in an increase of FA concentration up to 5 mg N L^{-1} during this phase of the reactor operation, which is also considered to be inhibiting for NOB [12,35]. However, the FA concentration did not exceed 1.5 mg N L^{-1} during all of Phase IV, while the maximum was registered at day 288 within the period with complete nitrification to N-NO_3^- (Fig. 3(B)). In addition, Rongsayamanont et al. [36] found that FA at concentrations between 4 and 9 mg L^{-1} N, as a sole factor, does not lead to the long-term suppression of NOB activity during the treatment of TAN-concentrated wastewater. As a consequence, the influence of FNA seems to be significantly stronger compared to the effects of FA under the conditions applied during this experiment.

3.5. Concentration of suspended solids

The average TSS concentration in the reactor during the experiment reached 8.6 ± 0.8 g L^{-1}, and the average sludge concentration expressed as VSS was 7.1 ± 1.7 g L^{-1} during the whole experimental period, except Phase I (the start-up process of the reactor).

3.6. Evaluation of NOE, NOR, and sNOR during the experiment

The changes of DO supply strategy significantly influenced not only the representation of the final nitrification products during the individual phases of reactor operation, but also the performance of TAN removal expressed by NOE and NOR. The average values of these parameters achieved within individual phases are summarized in Table 4.

In order to evaluate the variability of NOE and NOR during the individual phases, statistical analysis was performed. NOE shows a difference with a level of significance of $\alpha < 0.05$ between NOE in Phase II and III as well as in III and IV. NOR and sNOR show

Table 4
Reactor conditions and operation length of the experimental phases

Phase	NOE (%)	NOR (kg N m^{-3} d^{-1})	sNOR (mg N g VSS^{-1} d^{-1})
II	96.3 ± 1.7	1.37 ± 0.06	193 ± 9
III	71.4 ± 20.0	0.94 ± 0.28	133 ± 40
IV	93.5 ± 5.5	1.04 ± 0.15	147 ± 23

statistical differences between Phases II and III, III and IV, and II and IV. As a consequence of this finding, it is possible to conclude that DO limitation had a negative impact on the effectiveness of TAN removal under given conditions.

Considerably high NOR (1.12 kg TAN m^{-3} d^{-1} on average) was achieved steadily during the partial nitrification period of Phase IV. For example, under similar conditions (partial nitrification of TAN-concentrated wastewater in CSTR regime, controlled pH value, 25°C), Jubany et al. [22] reported a maximum NOR of 0.8 kg TAN m^{-3} d^{-1}. Blackburne et al. [17] applied limited DO conditions leading to 70% of nitrite accumulation with a maximum NOR around 0.25 kg TAN m^{-3} d^{-1} at a temperature between 19 and 23°C. The common NOR value for the SHARON process ranges between 0.5 and 0.8 kg TAN m^{-3} d^{-1} at significantly elevated temperature of 35°C [7]. The limits for NOR in the first two setups were probably based on continuous DO limitation, while the third process was limited by a short SRT, which would not allow feeding with higher NLR [37]. The high NOE levels reached during the phases without DO limitation (Phase III and IV) were enabled by pH control in accordance with Jenicek et al. [27].

The practical implementation of the observations presented in this paper could be based on switching between both phases (high and low DO) with the aim to achieve a high level of N-NO_2^- accumulation (application of temporary DO limitation) and maximum NOE and NOR (operating the reactor without DO limitation during the rest of operational time) at the same time. The evaluation of the optimal length for both phases is needed to be studied further.

3.7. Microbial communities—FISH analysis

The first sample was taken during Phase II (day 127) under full nitrification conditions, while the second analyzed sample was prepared in the beginning of Phase IV (day 204) under conditions with intensive nitrite accumulation (97.6%). The sample taken in Phase II contained a sufficient amount of both AOB and NOB biomass (20.4 and 6.4% from the total biomass) (Fig. 5(A)). More than ¼ of the total biomass was formed by nitrifiers, which indicates adaptation of the biomass in specific characteristics of reject water (low easily degradable COD, high TAN content) compared to a standard amount of nitrifying bacteria in common activated sludge between 3 and 10% [38]. The second sample (taken during Phase IV) shows a significant predominance of AOB (accounting for 27.2%) over NOB (below 1%) from the total biomass (Fig. 5(B)). The FISH results supplementary to the observations from regular physical–chemical analyses performed during the experiment confirm AOB predominance. The NOB ratio of total biomass went

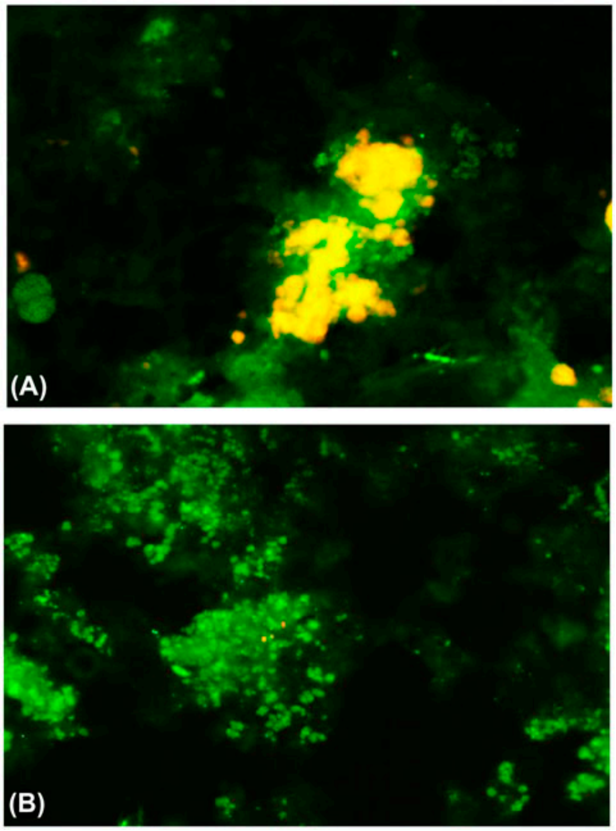

Fig. 5. Pictures from FISH, AOB are green colored, NOB yellow-red, enlargement 320 × (A) Sample taken during the Phase II and (B) sample taken during the Phase IV.

through significant decline between both measurements, confirming the assumption that NOBs were washed out during the period with DO limitation.

4. Conclusions

The importance of indirect effects of DO limitation on the long-term distribution of final products of nitrification applied for separate reject water treatment was confirmed in this study. The restriction of NOB activity primarily caused by DO limitation induced the increase of nitrite concentration, which subsequently further suppressed NOB growth through FNA inhibition. Even the termination of DO limitation after a period lasting 39 d did not result in the immediate increase of NOB activity, indicating the importance of FNA inhibition under given conditions. Another period without DO limitation lasting 100 d was needed for a complete recovery of NOB activity. With respect to the negative influence of DO limitation on the nitrogen oxidation rate (or more precisely on NOE), the presented findings could be used simultaneously for the control of nitrate production and for optimizing the process efficiency.

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