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The occurrence and behaviour of nitrite (NO₂) in full-scale biological nutrient removal wastewater treatment plants --Manuscript Draft--

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Abstract:	Nitrite is an important intermediate in nitrogen conversions in full-scale wastewater treatment plants (WWTPs). During field experiments, very small nitrite concentrations were measured (<0.1 gN/m ³) in the studied plants. However, a significant nitrite accumulation was observed during various laboratory experiments with the process mixed liquor. These results are important for determining appropriate values of kinetic parameters related to the nitrogen conversions in WWTPs.

The occurrence and behaviour of nitrite (NO₂) in full-scale biological nutrient removal wastewater treatment plants

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Abstract: Nitrite is an important intermediate in nitrogen conversions in full-scale wastewater treatment plants (WWTPs). During field experiments, very small nitrite concentrations were measured (<0.1 gN/m³) in the studied plants. However, a significant nitrite accumulation was observed during various laboratory experiments with the process mixed liquor. These results are important for determining appropriate values of kinetic parameters related to the nitrogen conversions in WWTPs.

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Introduction

The conventional method of biological nitrogen removal includes complete oxidation of ammonia (NH₄-N) to nitrate (NO₃-N) (nitrification) followed by the reduction of nitrate to dinitrogen gas (N₂) (denitrification). Nitrite (NO₂-N) is a common intermediate in these conversions and its accumulation in full-scale wastewater treatment plants (WWTPs) has been reported in literature for decades. The mechanisms of NO₂-N formation are complex with many competing and parallel conversions of production and consumption for the compound. Foley et al. (2010) noted that “*nitrite is simultaneously a product, a substrate and an inhibitor, which can be formed and utilised under both aerobic and anoxic conditions, by several different types of microorganisms*”. Partial nitrification (conversion of NH₄-N only to NO₂-N) has gained increasing attention in recent years due to some potential benefits, such as lower oxygen requirements, reduced COD demand in the follow-up NO₂-N reduction, lower biomass production, etc.

Under normal operating conditions, however, NO₂-N accumulation is undesired in biological nutrient removal (BNR) WWTPs. It has been reported that NO₂-N could inhibit P release and uptake by the polyphosphate accumulating organisms. The accumulation of NO₂-N has also been shown to trigger the emission of nitrous oxide (N₂O) during denitrification (Lemaire et al., 2008; Foley et al., 2010). During unstable operation of municipal WWTPs, e.g. due to insufficient DO concentration, low temperature, short sludge retention time or the presence of inhibitory compounds, NO₂-N can play an important role in the nitrogen conversions (Sin et al., 2008).

The aim of this study was to investigate the occurrence and behaviour of NO₂-N directly in full-scale WWTPs and during various experiments with the process biomass.

Material and Methods

The field and laboratory experiments were carried out at four BNR WWTPs of different size and process configuration. The field measurements comprised detailed influent and effluent characterization (10 analyses of composite samples) of nitrogen species (total N, NH₄-N, NO₃-N and NO₂-N) as well as concentration profiles inside the bioreactors. The profiles of the nitrogen species were developed based on the averages of three grab samples from the BNR process inlet (settled wastewater), anaerobic compartment, anoxic compartment and last aerobic compartment effluents. In one plant (Gdansk), more detailed measurements were also carried out inside the anoxic and aerobic compartments.

The laboratory experiments were carried out in an experimental set-up consisting of two parallel batch reactors ($V=4 \text{ dm}^3$) with an automated control system for temperature and DO concentration. Three kinds of kinetic batch experiments with the process mixed liquor were carried out, including one-phase (anoxic) and two-phase (anaerobic/anoxic) experiments with different carbon sources, and three-phase (anaerobic/anoxic/aerobic) experiments with the settled wastewater added at the beginning of the anaerobic phase. In the latter experiments, the fluorescence in situ hybridization (FISH) was used to identify the microbial groups responsible for nitrification, i.e. ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB).

In the additional experiments with reject water, the batch reactors (described above) were operated in a sequencing “fill-and-draw” mode for approximately four weeks. An acclimation period of the process mixed liquor to external carbon sources (fusel oil and ethanol) was investigated in terms of achieving the maximum denitrification rate.

Results and Discussion

During the field experiments, no significant $\text{NO}_2\text{-N}$ accumulation was observed in either bioreactors or secondary effluents. A detailed example of these measurements at the Gdansk WWTP is shown in Table 1 and Figure 1. In contrast, a complete $\text{NH}_4\text{-N}$ oxidation was not achieved in the batch reactors (three-phase experiments) and $\text{NO}_2\text{-N}$ was accumulated, reaching even the level of $8\text{-}9 \text{ gN/m}^3$ in the Gdansk WWTP (Figure 2). The apparent aerobic $\text{NO}_2\text{-N}$ production rates ranged from 0.12 to $0.75 \text{ gN/(kgVSS}\cdot\text{h)}$. The composition of AOB and NOB populations was only slightly affected by the plant characteristics. Within the AOB population, *Nitrosomonas* was detected with medium occurrence, and an occasional occurrence of *Nitrosococcus*. In all the experiments, $\text{NO}_2\text{-N}$ oxidation was catalysed primarily by *Nitrospira*-like bacteria, whereas *Nitrobacter* was detected only occasionally.

In one-phase experiments with the settled wastewater, $\text{NO}_2\text{-N}$ accumulations $>2 \text{ gN/m}^3$ occurred in the first phase of the test and $\text{NO}_2\text{-N}$ was subsequently utilized in the second phase (Figure 3a). However, external carbon sources, such as ethanol and fusel oil, had minor (or no) effects on the behaviour of $\text{NO}_2\text{-N}$ in the similar experiments (Figure 3b).

During the acclimation of the process biomass to fusel oil and ethanol, a significant enhancement of the denitrification efficiency was observed (Figure 4). The $\text{NO}_3\text{-N}$ utilization rates were continuously increasing from below $1 \text{ g N/(kg VSS}\cdot\text{h)}$ to over $15 \text{ g N/(kg VSS}\cdot\text{h)}$ over the entire operational period. This increase was accompanied by a significant ($>30 \text{ gN/m}^3$), but temporal, accumulation of $\text{NO}_2\text{-N}$ in the initial 2-3 hours of the anoxic phase (Figure 4b). Furthermore, after the 4-week acclimation period, higher $\text{NO}_2\text{-N}$ concentrations were also observed in the aerobic phase compared to the initial measurements with the non-acclimated biomass.

References

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Table 1. Measured concentrations of N species inside the bioreactor at the Gdansk WWTP (INF – influent, ANA- anaerobic zone, AX3 – last anoxic zone, AER6 – last aerobic zone).

Measurement	NH ₄ -N, g N/m ³				NO ₃ -N, g N/m ³				NO ₂ -N, g N/m ³			
	INF	ANA	AX3	AER6	INF	ANA	AX3	AER6	INF	ANA	AX3	AER6
Measur. 1	44.6	32.9	8.7	0.39	0.15	0.16	0.38	7.7	0.04	0.16	0.06	0.07
Measur. 2	47.8	34.4	8.5	0.23	0.11	0.15	0.26	7.2	0.03	0.03	0.04	0.08
Measur. 3	51.2	33.8	7.5	0.14	0.12	0.12	0.12	5.7	0.03	0.02	0.03	0.08
Measur. 4	60.2	37.0	7.0	0.05	0.13	0.1	0.09	4.8	0.01	0.02	0.01	0.05
AVERAGE	51.0	34.5	7.9	0.20	0.13	0.13	0.21	6.4	0.03	0.06	0.04	0.07

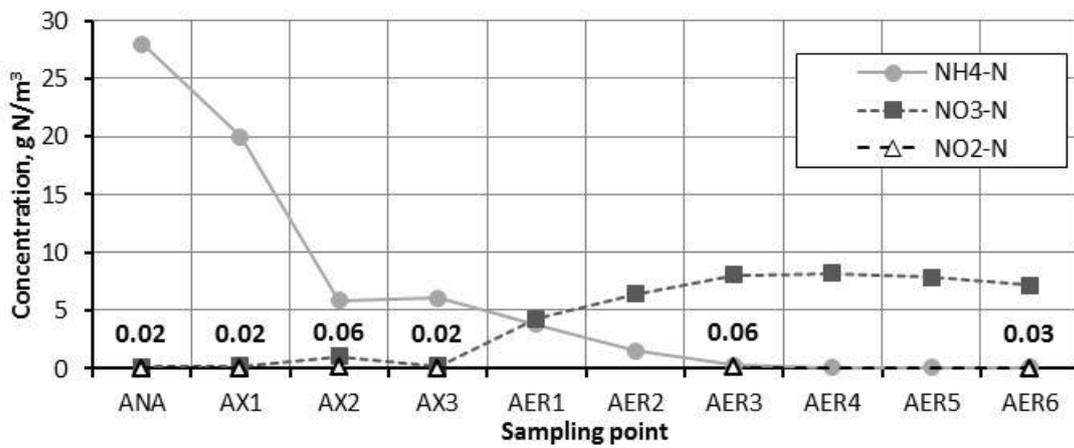


Figure 1. Measured concentration profiles of N species inside the bioreactor at the Gdansk WWTP (ANA- anaerobic zone, AX – anoxic zone, AER – aerobic zone).

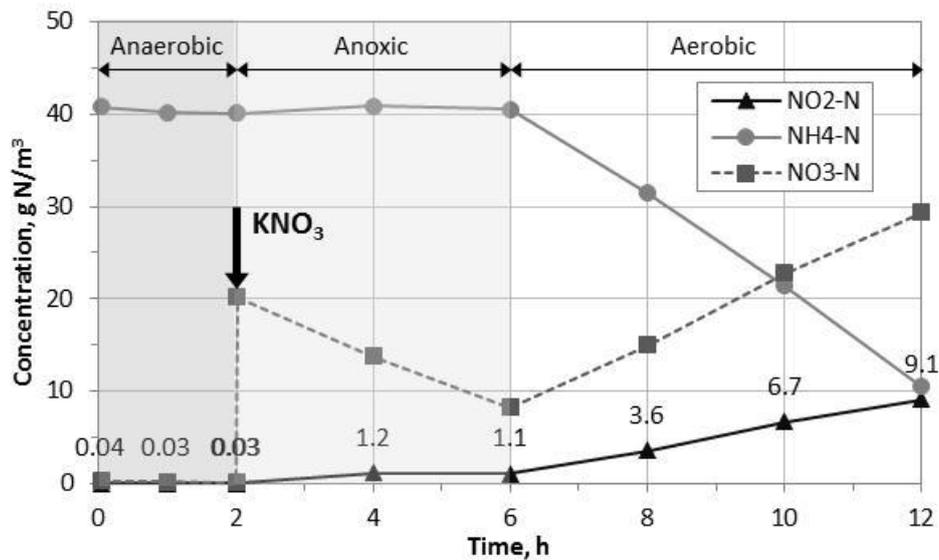


Figure 2. Measured concentrations of N species during a sample 3-phase batch experiment with the mixed liquor and settled wastewater at the Gdansk WWTP

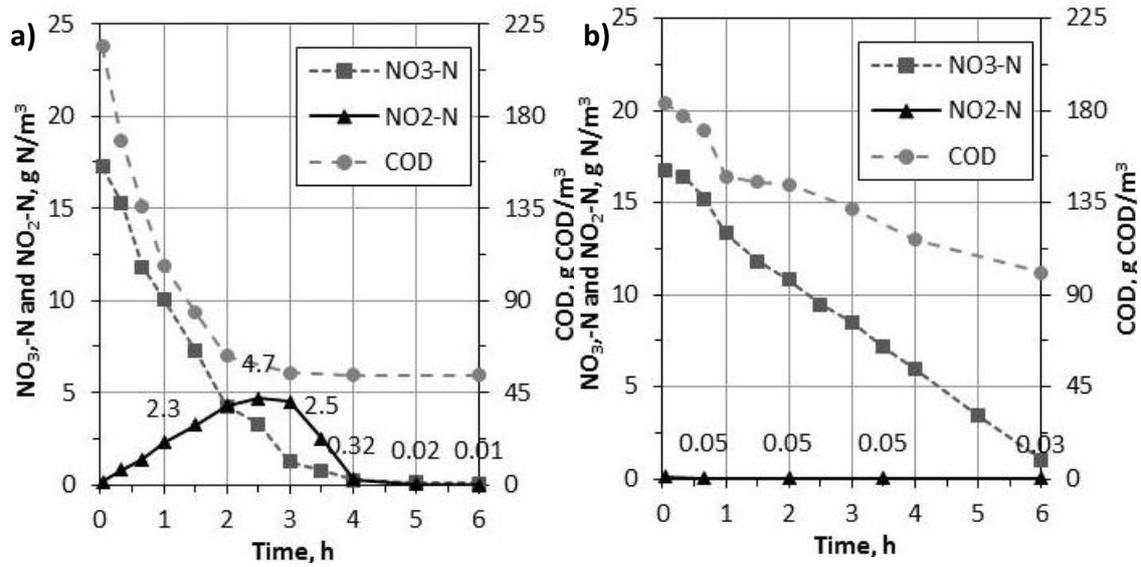


Figure 3. Sample results of the one-phase experiments with the settled wastewater (a) and fusel oil (b) in the lab-scale batch reactors (non-acclimated biomass)

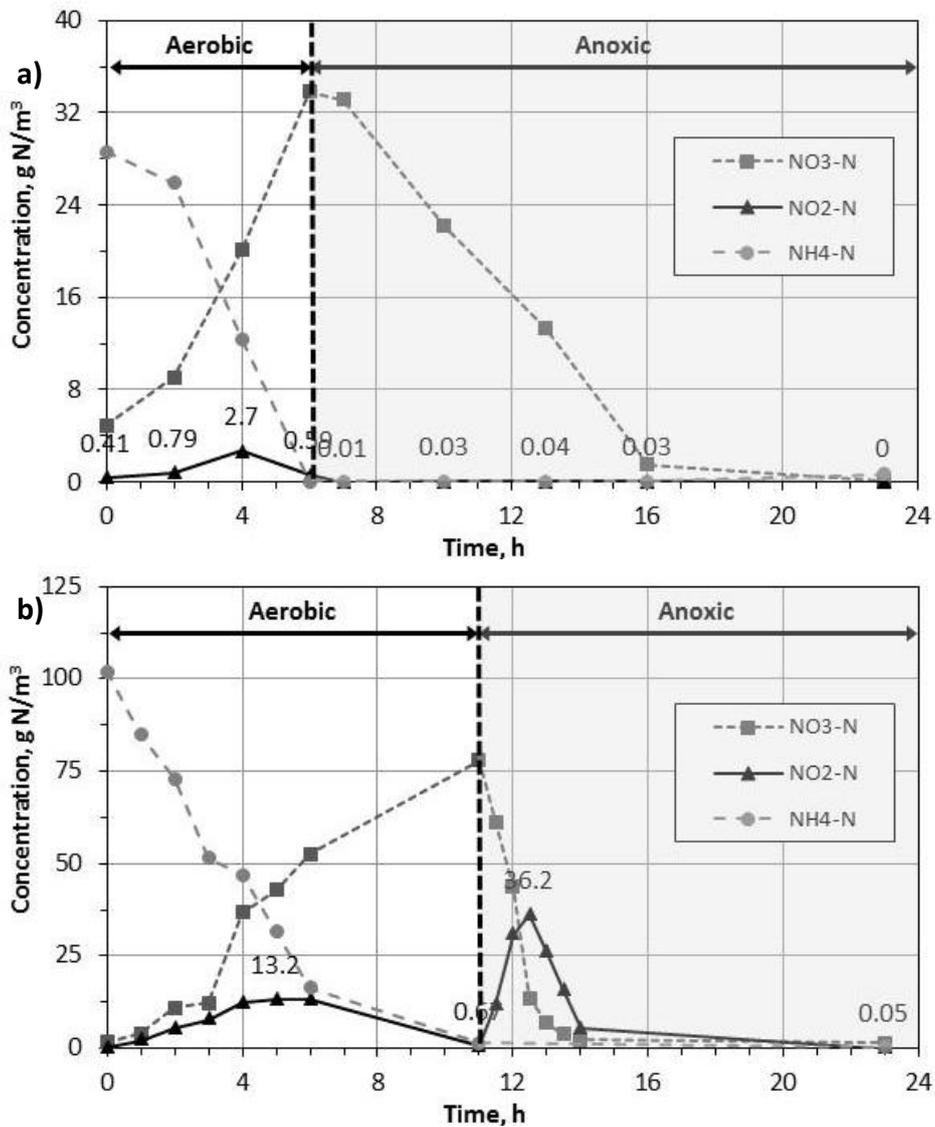


Figure 4. Sample behaviour of N species ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$) during acclimation of the process biomass to ethanol during reject water treatment: (a) non-acclimated biomass, (b) 4-week acclimation