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Activated sludge vs. biofilm – effect of temperature on ammonia and nitrite oxidation rate in the hybrid reactor

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ABSTRACT

Nitrification is considered one of the most temperature-sensitive biological steps in wastewater treatment. Nitrifying bacteria are highly sensitive to temperature drops, resulting in a rapid decrease in their activity. This study compares the effect of a rapid temperature decrease on the ammonia oxidation rate (AOR) and nitrite oxidation rate (NitOR), with consideration of the form in which biomass develops in the IFAS-MBSBBR. Ammonia Utilisation Rate Tests and Nitrite Utilisation Rate Tests were conducted for two temperatures, namely 20°C and 12°C, for the following forms of biomass: activated sludge (AS), biofilm (B), and combination of both – hybrid (H). The tests showed that nitrite oxidising bacteria inhabiting biofilm were more sensitive to a rapid temperature change than those in activated sludge. A sudden drop of temperature caused a 15% higher than predicted decrease in AOR for AS. At 12°C, AOR changed more considerably than NitOR in tests carried out for H. A temperature correction coefficient of 1.107–1.087 was proposed, applicable in hybrid wastewater treatment systems. Microbiological analysis shows that nitrifiers occurred more abundantly in biofilm than in activated sludge.

Keywords: Temperature correction coefficient; Ammonia oxidation rate; Nitrite oxidation rate; IFAS; Activated sludge; Biofilm

1. Introduction

The nitrification process is one of the primary links of the nitrogen cycle in the environment. It has been long known as one of the most temperature-sensitive steps of biological wastewater treatment. Low temperature particularly strongly affects the microbial metabolic rate, generally expressed as the rate of growth or absorption, resulting in a decrease in the efficiency and rate of the nitrification process [1]. Research has shown that a rapid change in temperature may prevent proper functioning of bacterial proteins by damaging the cell's outer membrane and/or turning it gel-like [2], leading to a decrease in the rate of transfer of substrate, including oxygen. According to Beagles et al. [3], the magnitude of the effect of cold shock depends on the temperature gradient, cooling rate, state of the culture medium, and strain of microorganisms. According to the cited authors, a decrease in temperature is accompanied by an extended lag phase before growth, a decrease in the growth rate, and a potential decrease in the final abundance of cells.

In many countries around the globe, seasonal conditions related to seasons of the year affect the temperature of wastewater. It varies from below 10°C in winter to more than 20°C in summer [4]. It is therefore important that the processes used for wastewater treatment are effective in the context of seasonal temperature fluctuations. The design and

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operation of objects responsible for wastewater treatment should therefore consider temperature variability, particularly affecting the lifespan of nitrification bacteria. Nitrifying bacteria are microorganisms extremely sensitive to temperature decreases, contributing to a rapid reduction of their activity. Many papers published to date focus on the general rate of the nitrification process, although ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) have different growth and temperature correction coefficients [5].

Published studies show that the effect of a rapid decrease in temperature may depend on the form in which biomass develops in the reactor [5–8]. The nitrification rate at the moment of a rapid temperature decrease from 20°C to 10°C determined by Hwang and Oleszkiewicz [5] was 20% lower than predicted by the suggested temperature correction coefficient for a system operating in the active sludge technology. Ahmed et al. [6] investigated the effect of a rapid temperature decrease from 10°C to 1°C in a moving bed biofilm reactor (MBBR). The nitrification rates measured by the authors were on average 21% higher than those obtained with the application of Arrhenius correction coefficients of 1.09 and 1.086. The cited authors focused on the effect of temperature on particular forms of biomass developing in wastewater treatment systems. It is interesting, whether a given form of biomass will react to changes in temperature the same way when it cooperates with another in a single reactor. It should be emphasised that hybrid systems with two cooperating forms of biomass are gaining popularity in wastewater treatment plants.

Information regarding a rapid temperature decrease in hybrid systems, however, is hardly available. The issue was addressed in own research by comparing the effect of a rapid temperature decrease on the rate of particular stages of the nitrification process in an integrated fixed-film activated sludge – moving bed sequencing batch biofilm reactor (IFAS-MBSBBR). It permitted the determination of the dependency of the activity of particular groups of nitrifying microorganisms in a hybrid system on sudden short-term temperature fluctuations, as well as on the form of developing biomass. Moreover, next generation sequencing was used to identify the nitrifiers in activated sludge and biofilm, and to monitor changes in their abundance. Knowledge regarding the nitrifiers offers better understanding of the impact of a rapid temperature decrease on the nitrification process.

2. Methodology

Batch tests for the determination of the effect of temperature on the activity of particular groups of nitrifying microorganisms were conducted for biomass from the laboratory model of IFAS-MBSBBR with an active volume of 28 L for integrated carbon, nitrogen, and phosphorus removal by means of classic nitrification/denitrification (N/D) (Fig. 1). Moving bed EvU-Pearl with cylindrical shape with corrugated external and internal surface with dimensions of Φ = 5 mm, h = 8 mm was used as a biomass carrier with an active surface of 600 m^2/m^3. The moving bed constituted 25% of the active volume of the reactor (amount of biofilm – approximately 0.28–0.29 $gTSS/m^2$). The concentration of activated sludge was maintained at a level of 1.2–1.4 gMLSS/L. The hydraulic retention time (HRT) and sludge retention time (SRT) were 0.93 h and 20 d, respectively.

Constant temperature at a level of 20°C was maintained in the reactor by means of an external air conditioning system. The system operated in three 8-h cycles/d, involving the following subsequent phases: I phase without aeration – 50 min, I aerobic phase (with intermittent aeration – 20 min with aeration (t_1); 10 min without aeration (t_2); $R = t_2/t_1 = 1/2$) – 190 min, II phase without aeration – 30 min, II aerobic phase (with intermittent aeration – 20 min with aeration; 10 min without aeration; R = 1/2) – 150 min, sedimentation – 50 min, decantation – 10 min. During the aerated phases, the oxygen concentration was maintained at a level of 1.5 mgO_2/L.

At the beginning of each phase without aeration, raw wastewater was dosed to simulate the composition of municipal sewage, prepared based on dechlorinated tap

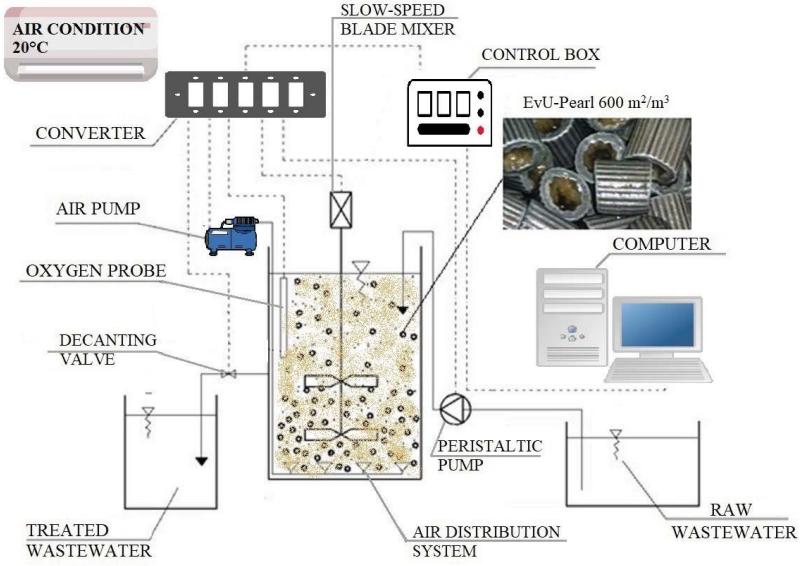

Fig. 1. Schematic of reactor with accessories.

water and a mixture of: ammonium acetate 225 mg/L; peptone 135 mg/L; starch 45 mg/L; glucose 45 mg/L; glycerine 0.049 ml/L; $NaHCO_3$ 125 mg/L; Na_2HPO_4 15 mg/L; KH_2PO_4 4.5 mg/L in a volume of 10 L per cycle.

The experiment determining the effect of temperature on the nitrification process in a hybrid reactor involved conducting the tests in two repetitions. The first one (1st rep.) was carried out after 14 d of stable operation of the system under the conditions described above. The second one was preceded by a period of introduction of changes in the methodology of operation of the reactor due to the outbreak of the global Covid-19 pandemic. The changes included reduction of the reactor's organic (L_{COD}) and nitrogen (L_N) loading rate (from L_{COD} = 536 gCOD/m^3·d, L_N = 64 gN/m^3·d to L_{COD} = 402 gCOD/m^3·d, L_N = 48 gN/m^3·d) through a decrease in the volume of raw wastewater supplied to the system. No experiments were conducted during that time – stable operation of the system was only maintained. With the removal of restrictions, the assumptions arranged at the beginning of the experiment were restored, and after 14 d of stable work of the reactor, a second repetition (2nd rep.) was implemented.

The tools applied for the determination of the activity of particular groups of nitrifying microorganisms were Ammonia Utilisation Rate Tests (AUR) and Nitrite Utilisation Rate Tests (NitUR). The tests were conducted for two temperatures, namely 20°C and 12°C, for the following forms of biomass: activated sludge (AS), biofilm (B), and combination of both – hybrid (H). During the tests, oxygen concentration was maintained at a level of 1.5 mgO_2/L.

Before launching the tests, biomass sampled from IFAS-MBSBBR was washed with dechlorinated tap water to remove N–NH_4^+, N–NO_2^-, N–NO_3^-, and then placed in a test reactor with a volume of 14 L, keeping the proportions of biomass the same as those in the main reactor. The reactor was filled to 13.9 L with dechlorinated tap water, previously heated or cooled to 20°C or 12°C, respectively, and deoxidised to a level of 1.5 mgO_2/L. Using thermostatic water bath (F32-ME Refrigerated/Heating Circulator, JULABO GmbH), the set temperature was maintained during particular tests. The DO concentration and temperature was controlled using a Memosens Optical Oxygen Sensor COS81D (Endress + Hauser, Germany) cooperating with the automatic system.

The test began with dosing 4% solution of NH_4Cl (AUR) or 5% solution of KNO_2 (NitUR) to the test reactor, to an amount ensuring obtaning the assumed concentration of N–NH_4^+ or N–NO_2^- of 15 mg/L. An important methodological assumption in the experiment was introducing the appropriate amount of 5% solution of $KHCO_3$ to avoid alkalinity being a factor limiting the course of the nitrification process. Considering the theoretical comment, for the value required for alkalinity for nitrification of 7.14 mg$CaCO_3$/mgN–NH_4 [9], the initial alkalinity value was adopted at a level of 200 mg$CaCO_3$/L.

Throughout the experiment, the reactor was aerated by means of an aeration system consisting to a blower and aquarium filters mounted on the bottom of the reactor. The content of the reactor was stirred by means of a slow-speed blade mixer R-50D by CAT with a rotation speed of approximately 110 rpm. Every 60 or 30 min, samples with a volume of 30 mL were collected from the reactor and immediately filtered through 0.45 μm mesh. Concentration of the following was determined in the filtrate: N–NH_4^+, N–NO_2^-, N–NO_3^- (AUR test), N–NO_2^-, and N–NO_3^- (NitUR test). The test lasted until concentration of N–NH_4^+ (AUR test) or N–NO_2^- (NitUR test) decreased to a value approximate to 0 mg/L, or until the concentration of the indicator was maintained at a comparable level for subsequent 60 min. In the case of a test performed for activated sludge and hybrid, mixed liquor volatile suspended solids (MLVSS) concentrations were also determined. In the case of tests with the application of carriers, the biomass amount (as volatile suspended solids) developed on the carriers was also determined. Concentrations of N–NH_4^+, N–NO_2^-, and N–NO_3^- were analysed spectrometrically according to APHA Standard Methods [10] using cuvette tests (Hach Lange GmbH) and a DR 3900 spectrophotometer (Hach Lange GmbH, Berlin, Germany). Mixed liquor volatile suspended solids (MLVSS) were determined using gravimetric methods in accordance with the Polish standard PN-EN 872:2007. Volatile suspended solids (VSS) in biofilm were also measured in accordance with Polish standard by calculation of weight loss. The biofilm was mechanically removed from the carries. All analyses were performed in duplicates.

The determination of the ammonia oxidation rate (AOR) or nitrite oxidation rate (NitOR) employed a straight-line fragment of the function of change in ammonia/nitrite concentration in time characterised by the coefficient $R^2 \geq 0.97$. The rate was expressed in mgN–NH_4^+ per (gVSS·h) (AUR test) or in mgN–NO_2^- per (gVSS·h).

On the date of conducting batch tests at a temperature of 20°C, samples of activated sludge and biofilm were collected for microbiological analyses.

DNA was isolated from the activated sludge and biofilm samples using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA). The isolation followed the instructions attached to the kit. The Qubit fluorometer (Invitrogen, USA) was used to quantify the isolated DNA. The isolated DNA was stored at –18°C until further analysis. The determination of the taxonomic composition of the analysed biomass samples involved sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene. High-throughput Illumina sequencing was performed with S-d-Bact-0341-b-S-17 and S-d-Bact-0785-a-A-21 primers [11] and NEBNext®High-Fidelity 2X PCR Master Mix (Bio Labs inc., USA) following the manufacturer's manual. Paired-end sequencing was performed on a Miseq sequencer with a MiSeq Reagent Kit V2 (Illumina, USA). The read length was 250 base pairs.

QIIMEII [12] package was used for the analysis of raw sequencing data. Pairs of sequences were merged using the fast-join algorithm. Unmerged sequences were excluded from further analysis. The Cutadapt algorithm was used to filter out low quality sequences (under 20) [13]. Chimeric sequences were detected and excluded from analyses using USEARCH [14]. 16S rRNA OTUs were picked from the Illumina reads using a closed-reference OTU picking protocol against the SILVA_V_138 database [15]. Sequences were clustered at 97% identity and trimmed to span only the 16S rRNA V4 region flanked by the sequencing primers. Taxonomy assignments were associated with OTUs based on the taxonomy associated with the SILVA_V_138 reference sequence defining each OTU.

3. Results and discussion

3.1. Identification of nitrifying bacteria

In both repetitions, batch tests for the determination of the effect of a rapid temperature decrease on the rate of particular stages of the nitrification process were preceded by the identification of nitrification microorganisms developed in the activated sludge and biofilm from IFAS-MBSBBR, and nitrification efficiency in this reactor were identified.

The results of 16S rRNA gene sequencing indicated the presence of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) in both forms of biomass from IFAS-MBSBBR. Among AOB bacteria, *Nitrosomonas* was the most abundant genus. Several unidentified genera of the *Nitrosomanadaceae* family were also found. The most abundant genus in the NOB community was *Nitrospira*. Less abundant *Nitrolancea* and *Candidatus Nitrotoga* were also identified.

Changes in the abundance of biofilm and activated sludge nitrifiers in samples from the first and second repetition are presented in Fig. 2. Nitrifiers were generally more abundant in biofilm than in activated sludge, both in the samples from the first and second repetition. The only genus that was more abundant in activated sludge than in biofilm was *Candidatus Nitrotoga*. In general, in all tested samples, the abundance of NOB bacteria was significantly higher than that of AOB. Between the first and second repetition, significant changes in the nitrifier community were observed, probably caused by the reduction of the organic and nitrogen loading rate prior to the second repetition. The change in the amount of incoming wastewater had a stronger impact on the nitrifying community developing in the biofilm, resulting in an increase in AOB abundance from 0.5% to 1.1%, and a decrease in NOB abundance from 5.7% to 2.5%.

NOB were much more abundant than AOB in the studied samples, unlike in the theoretical, thermodynamic model, assuming that AOB should be the dominant group the vast majority of NOB in the studied samples, however, were *Nitrospira*. *Nitrospira* is traditionally classified as NOB, although bacteria of the genus have been proven to also be capable of complete nitrification (comammox). Growth of comammox bacteria could disrupt the theoretical AOB/NOB ratio. Unfortunately, based on sequencing of the 16S rRNA gene, it is not possible to determine which fraction of the *Nitrospira* population in the tested samples is canonical *Nitrospira* and which is comammox. Another explanation for this disproportion may be the nitrate loop [16]. In this case, nitrite for NOB is supplied not only by AOB but also by denitrifiers detected in the analysed samples (*Denitratisoma* sp., *Dokdonella* sp., *Thauera* sp., *Rhodobacter* sp., *Zoogloea* sp.).

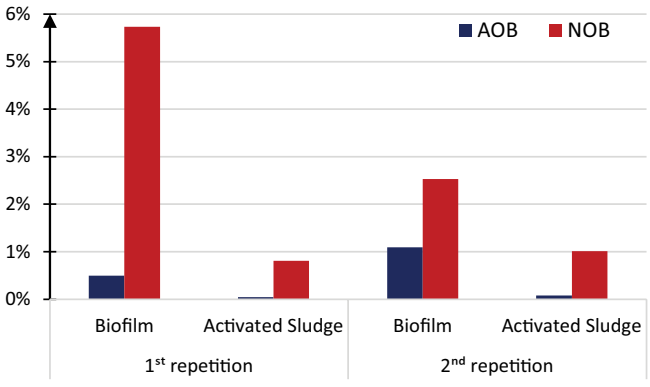

Fig. 2. Changes in the abundance of biofilm and activated sludge nitrifiers. Samples for new generation sequencing were taken from the main reactor working in 20°C. Graphs show the percentage contribution of AOB and NOB among all identified taxa.

In both repetitions, in the period of sample collection (used for the batch tests – chapter 3.2.), the efficiency of the nitrification process carried out at 20°C in IFAS-MBSBBR was comparable – 88.93% ± 4.54%. The concentration of ammonia nitrogen in the effluent was below 1.75 mg $N–NH_4^+/L$.

3.2. Effect of temperature on ammonia oxidation rate and nitrite oxidation rate

Batch tests for the determination of the ammonia oxidation rate (AOR) and nitrite oxidation rate (NitOR) for each of the analysed forms of biomass were conducted at two temperatures, namely 20°C and then 12°C, to show how a short-term temperature shock affects the activity of particular groups of nitrifying microorganisms depending on the form in which they develop in the reactor (Fig. 3). Results of particular batch tests are presented in the supplementary material.

As already observed earlier [5–7], a rapid decrease in temperature largely affects the kinetics of nitrification for suspended and attached biomass. Also in this study, for each of the designated forms of biomass, both in the case of AOR and NitOR values, at least 22% (1st rep.) and 27% (2nd rep.) differences were respectively recorded, compared to the analysed indices for 20°C and 12°C. Due to a rapid decrease in temperature, the ammonia oxidation rate decreased by 22% (1st rep.) and 57% (2nd rep.) for activated sludge, and by 57% (1st rep.) and 38% (2nd rep.) for biofilm, respectively. Batch tests for activated sludge from an industrial wastewater treatment plant by Gnida et al. [17] showed that the temperature can cause a significant decrease in the ammonia oxidation rate. According to the cited authors, the efficiency of the analysed process at 13°C decreased by up to 75% compared to that at 22°C. This is more than 3.41 (1st rep.) and 1.32 (2nd rep) times more than in our study for activated sludge, even though the temperature difference was only 1°C higher.

The obtained results also suggest that although the reactor operated in identical conditions, in the period preceding the values recorded for the second repetition, changes in the population of ammonia oxidising microorganisms could have occurred in IFAS-MBSBBR biomass, affecting their abundance or activity, and resulting in a different response of biomass to temperature changes. The results of the microbiological analysis, however, showed an increase in the amount of AOB bacteria, suggesting that the changes observed in AOR resulted from a much lower activity of microorganisms inhabiting the activated sludge flocs and biofilm at that time.

After a rapid temperature change, a greater difference in NitOR values was recorded each time for biofilm. In comparison to the recorded NitOR-AS, they were 1.32 (1st rep.) and 1.16 (2nd rep.) times higher, providing the basis for the

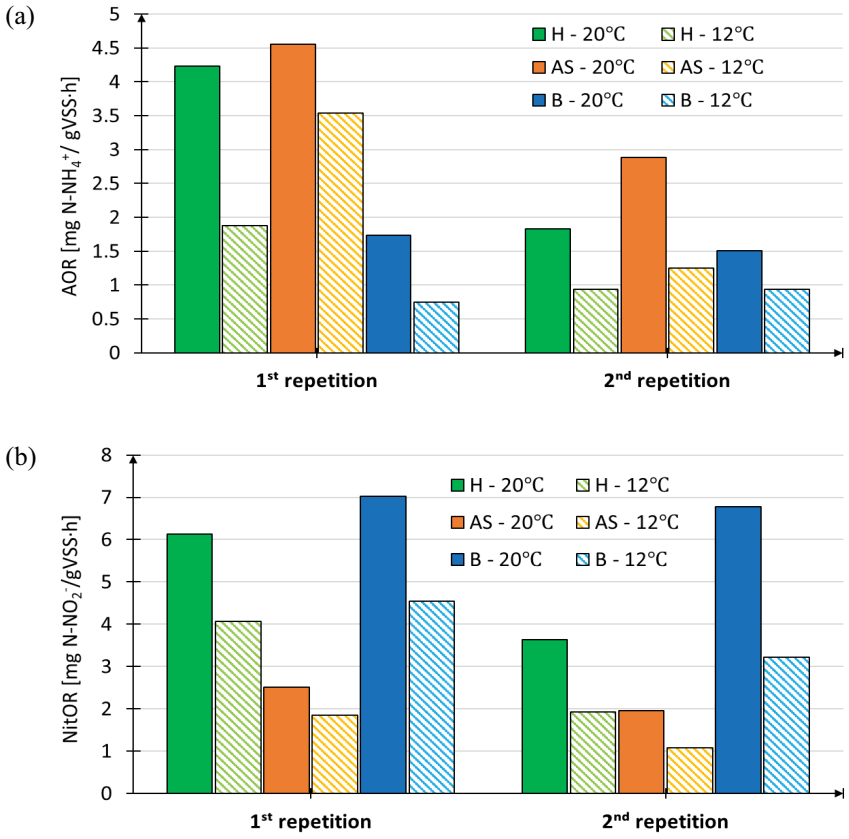

Fig. 3. Ammonia oxidation rate (AOR) (a) and nitrite oxidation rate (NitOR) (b) for hybrid (H), activated sludge (AS), and biofilm (B) at 20°C and 12°C.

conclusion that greater sensitivity to a decrease in temperature was shown for NOB developing in the hybrid reactor in the form of biofilm growing on moving carriers. According to Barria et al. [18] and Phadtare et al. [19], rapid temperature decreases caused disturbances in the basic cell functions, because they disturbed the mechanisms of transcription and translation, and resulted in greater membrane stiffness, and therefore lower substrate absorption and limitations of the function of transport through the membrane.

During tests conducted for both forms of biomass simultaneously cooperating at 12°C, greater differences were recorded in ammonia oxidation rates. They were 56% (1st rep.) and 49% (2nd rep.) lower than those conducted before a decrease in temperature. During the first repetition, differences in AOR and NitOR values between 20°C and 12°C slightly differed from those calculated for biofilm, whereas in the second repetition, they were approximate to those for sludge. It is also worth emphasising that the values of ammonia oxidation rate at 12°C for hybrid and biofilm in the second repetition were comparable, and approximately 1.33 times lower than AOR for activated sludge. A similar dependency was observed for NitOR values during the first repetition.

3.3. Temperature correction coefficients for IFAS-MBSBBR

The literature has already analysed the accuracy of application of the temperature correction coefficient in the nitrification process for systems based on activated sludge and biofilm [5,6,20,21]. No studies have been found, however, that would suggest how to refer the effect of temperature correction coefficients to the hybrid system.

Fig. 4 presents values of ammonia oxidation rate for activated sludge (AS) and biofilm (B) operating separately at 12°C, and values expected in calculations based on values of the discussed coefficients already provided in earlier studies in accordance with the Arrhenius equation (eq 1). The expected AOR values were calculated for the lower and upper range of the temperature correction coefficient provided by Hwang and Oleszkiewicz [5] (Ö = 1.072-1.127) and Salvetti et al. [22] (Ö = 1.023–1.081), respectively in the case of activated sludge and biofilm.

$$\mathrm{AOR}_{T2} = \mathrm{AOR}_{T1} \cdot \ddot{\theta}^{(T_2 - T_1)} \quad (1)$$

where AOR_{T1} and AOR_{T2} represent ammonia oxidation rate (mgN–NH_4^+/gVSS·h) at T_1 = 20°C and T_2 = 12°C, and Ö the temperature correction coefficient.

In accordance with the Arrhenius equation, adopting values of 1.072 or 1.127 as the temperature correction coefficient for activated sludge in the nitrification process should decrease the rate of ammonia oxidation by 2.612 mgN–NH_4^+/gVSS·h (1st rep.), 1.655 mgN–NH_4^+/gVSS·h (2nd rep.), or 1.750 mgN–NH_4^+/gVSS·h (1st rep.), 1.109 mgN-NH_4^+/

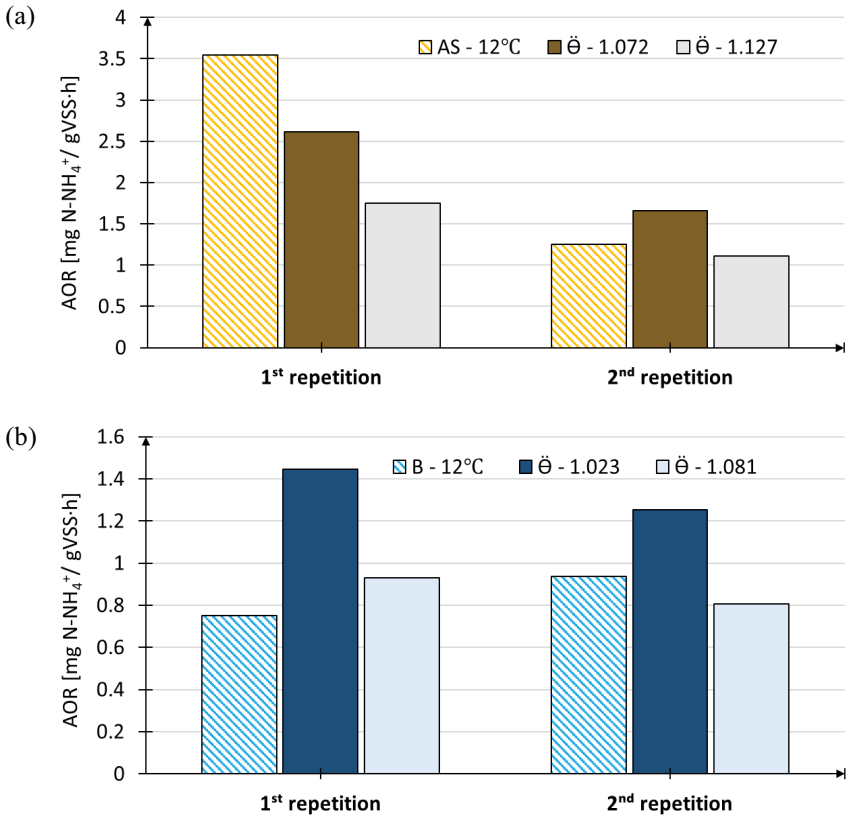

Fig. 4. Temperature correction coefficients for (a) activated sludge (AS) and (b) biofilm (B) in comparison to literature data.

gVSS·h (2nd rep.), respectively. In this study, the AOR values for activated sludge at 12°C during the first repetition were respectively 1.36 and 2.02 times higher than those proposed by Hwang and Oleszkiewicz [5]. The resulting temperature correction coefficient was 1.032. It was lower than values presented by Hwang and Oleszkiewicz [5]. The research of the cited authors, however, only covered a system with activated sludge. In this study, activated sludge was collected from a hybrid reactor where next to suspended biomass, nitrifying bacteria also develop on the biofilm. It is important, however, that in the second repetition, the AOR value observed in the study for 12°C was 0.75 times lower than that predicted after the application of the coefficient most frequently applied when designing a wastewater treatment plant. The ammonia oxidation rate is assumed to decrease by almost 43% with a decrease in temperature by approximately 8°C. This example, however, it does not concern a rapid change in temperature for sludge from a hybrid reactor in the case of which the shock related to low temperature caused a decrease in the AOR value by as much as 57%. Similar conclusions were drawn by Hwang and Oleszkiewicz [5], who obtained a decrease in specific nitrification rate 20% higher than predicted when using the current default correction factor, during a rapid change in temperature from 20°C to 10°C.

In the case of reactors operating in the moving bed technology, the temperature correction coefficient for biofilm under oxygen limiting conditions ranged from 1.023 to 1.081 (average value = 1.058) [22]. In the first repetition, the AOR value at 12°C was 32% lower than predicted after the application of the average value of the correction coefficient proposed by Salvetti et al. [22], and in the second repetition they were almost identical. The temperature correction coefficient calculated based on data obtained in the first repetition is 1.110 which is slightly higher than the upper range proposed by the cited group of researchers.

Over the recent years, an increasingly popular solution applied in wastewater treatment plants has been a technology based on the combination of activated sludge and biofilm on moving or fixed carriers. It allows for a considerable increase in the amount of biomass in the reactor in comparison to systems operating only with activated sludge, therefore permitting obtaining high efficiency of wastewater treatment at a substantially lower volume of the biological reactor [23]. Biofilm carriers also provide for longer biomass retention time (SRT), favouring the development of slow-growing nitrifiers, therefore increasing the efficiency of the nitrification process [24]. Based on results obtained in this study, and equation proposed by Arrhenius, it was attempted to determine the temperature correction coefficient for the hybrid system IFAS-MBSBBR. In the first repetition it was 1.107, and in the second one 1.087. The obtained values differ from the values proposed by Hwang and Oleszkiewicz [5] for activated sludge only in 1.4% and 1.8%. This suggests that nitrifiers overgrowing activated sludge flocs are of greater importance than those in biofilm

in the hybrid system. The values of temperature correction coefficient for biofilm were from 2.3% to 5.9% lower than those calculated for hybrid.

The direction of further research will be to determine the long-term impact of temperature changes on the nitrification process in a hybrid reactor.

4. Conclusions

- Nitrifiers were more abundant in biofilm than in activated sludge.
- NOB were much more abundant than AOB in the studied samples of activated sludge and biofilm.
- NOB inhabiting biofilm from IFAS-MBSBBR were more sensitive to a rapid temperature change than those in activated sludge.
- A sudden drop of temperature caused a 15% higher than predicted decrease in the ammonia oxidation rate for activated sludge.
- A sudden drop in temperature resulted in a greater change in ammonia oxidation rate values than those of nitrite oxidation rate in tests carried out for hybrid.
- The experiment provided the basis for proposing a temperature correction coefficient of 1.107–1.087, applicable in hybrid wastewater treatment systems.

Acknowledgments

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Supplementary information

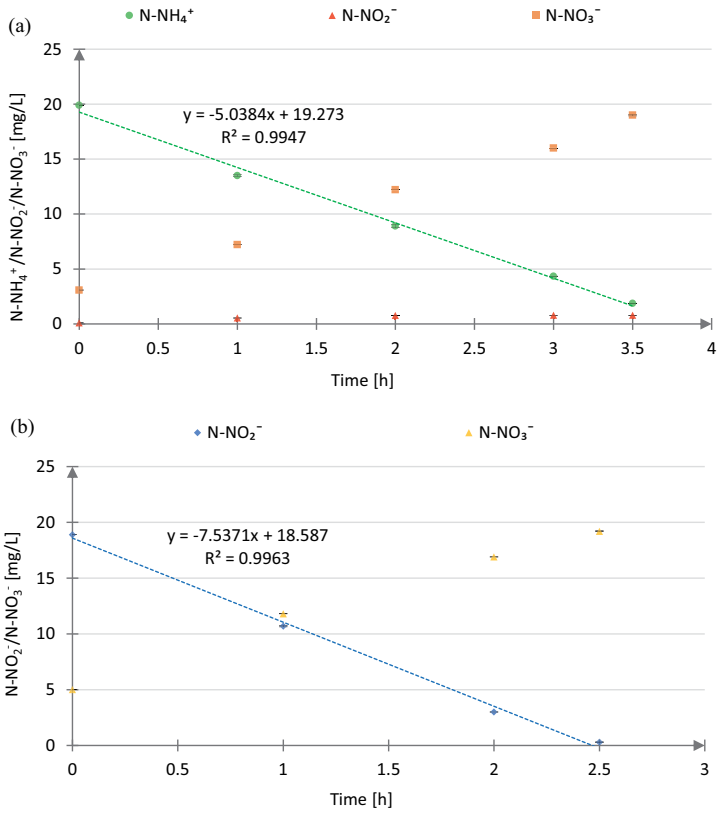
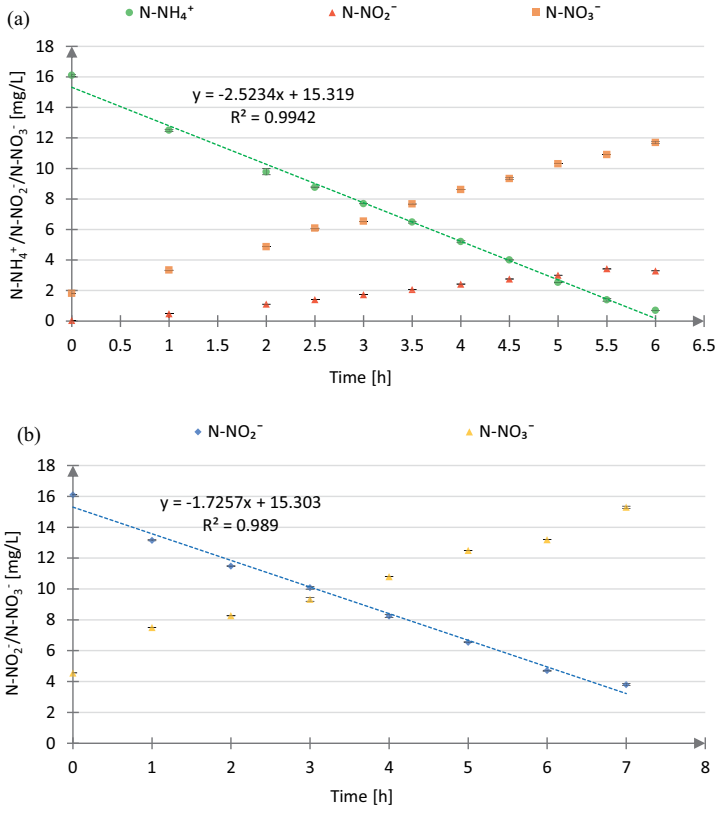


Fig. S1. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-H and (b) NitUR-H. 1st repetition – 20°C.



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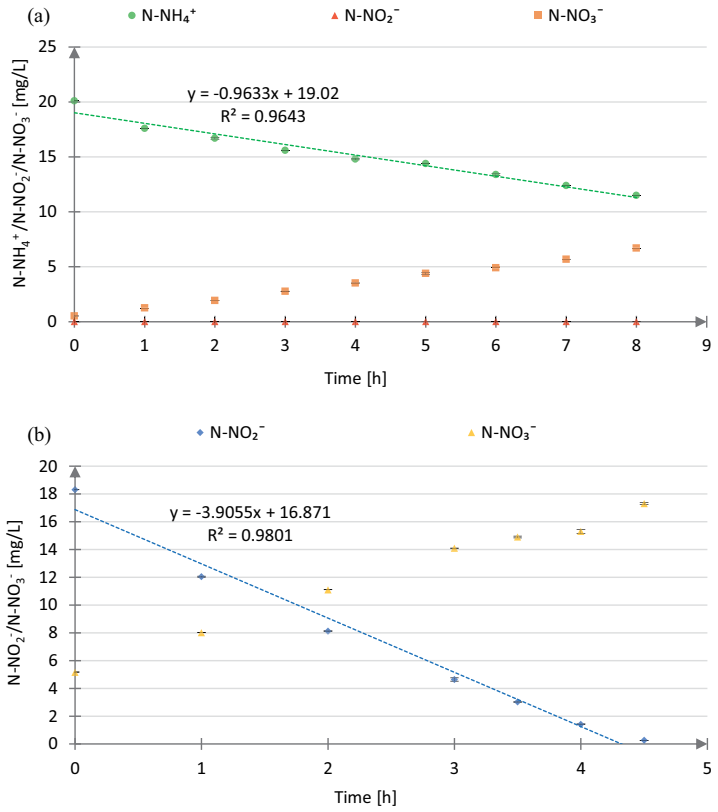

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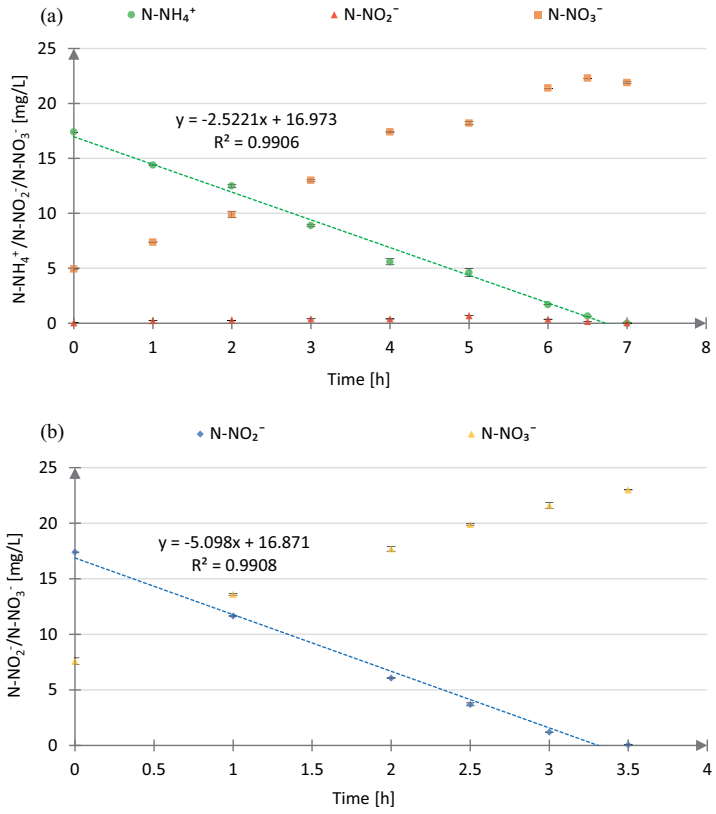

Fig. S4. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-H and (b) NitUR-H. 2nd repetition – 20°C.

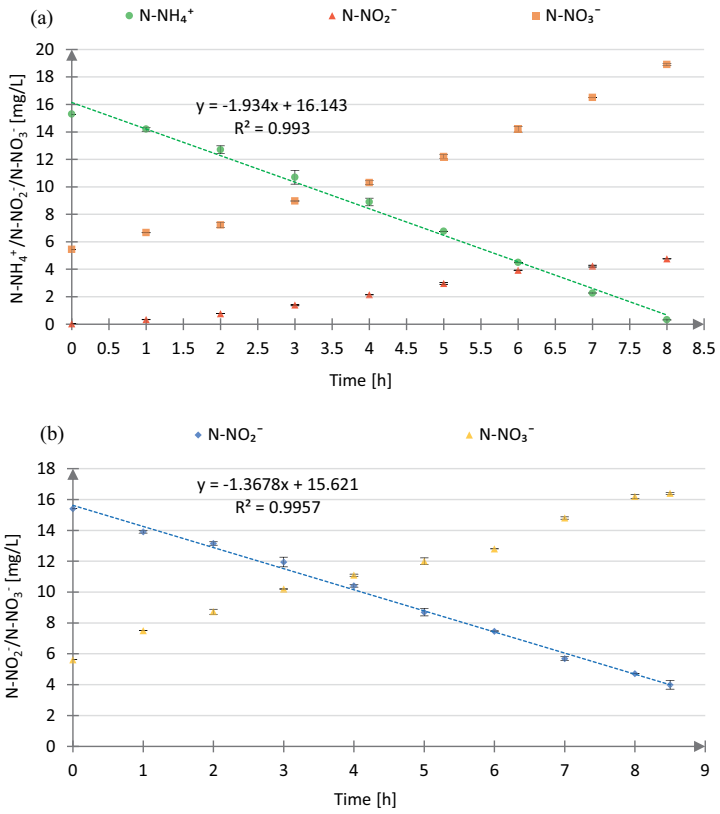

Fig. S5. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-AS and (b) NitUR-AS. 2nd repetition – 20°C.

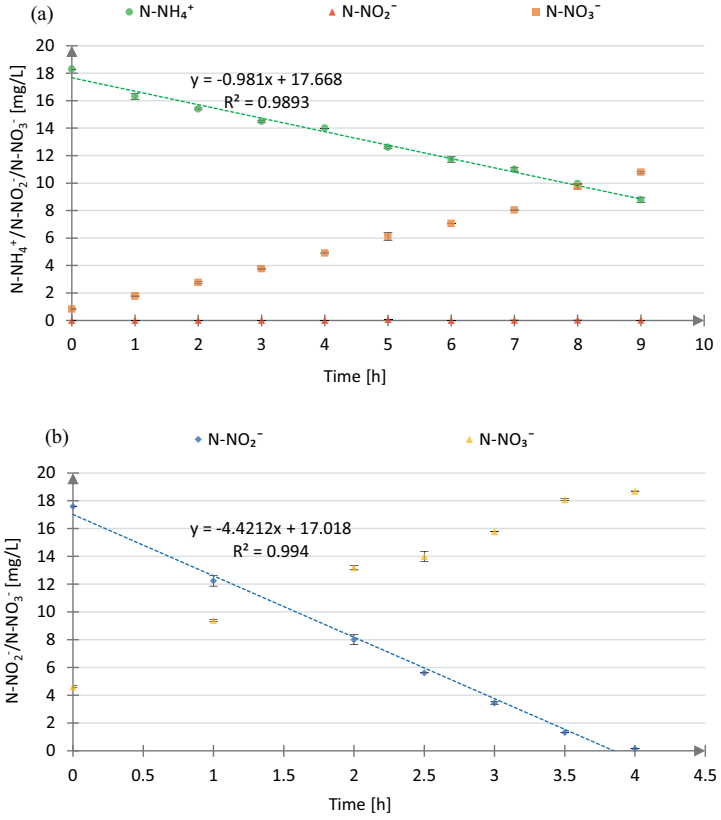

Fig. S6. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-B and (b) NitUR-B. 2nd repetition – 20°C.

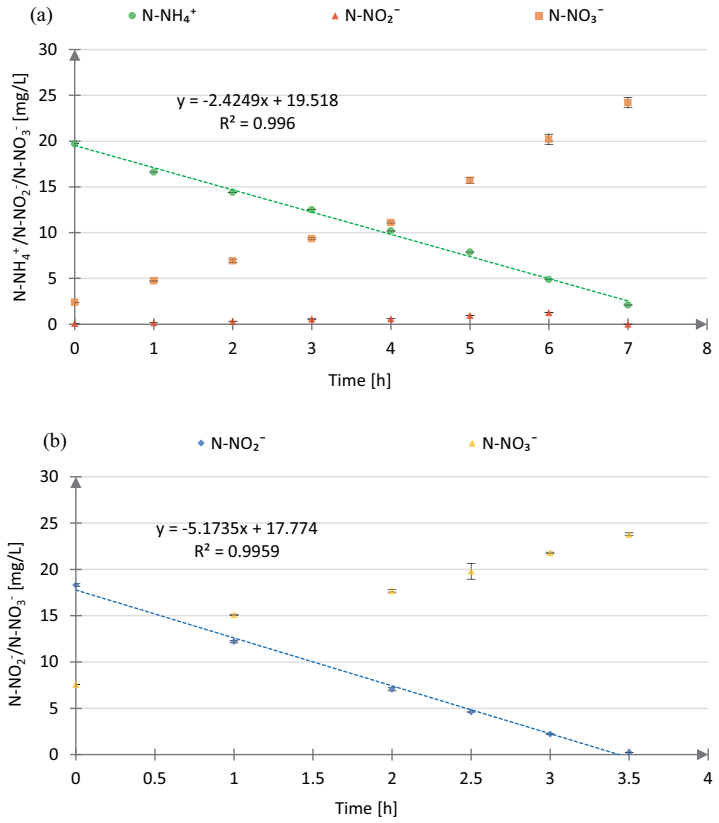

Fig. S7. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-H and (b) NitUR-H. 1st repetition – 12°C.

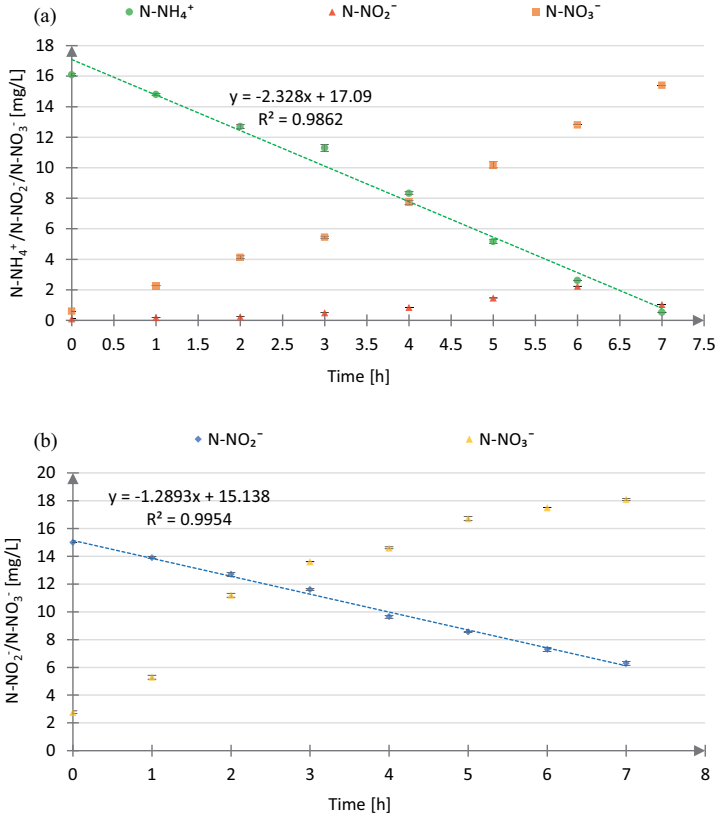

Fig. S8. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-AS and (b) NitUR-AS. 1st repetition – 12°C.

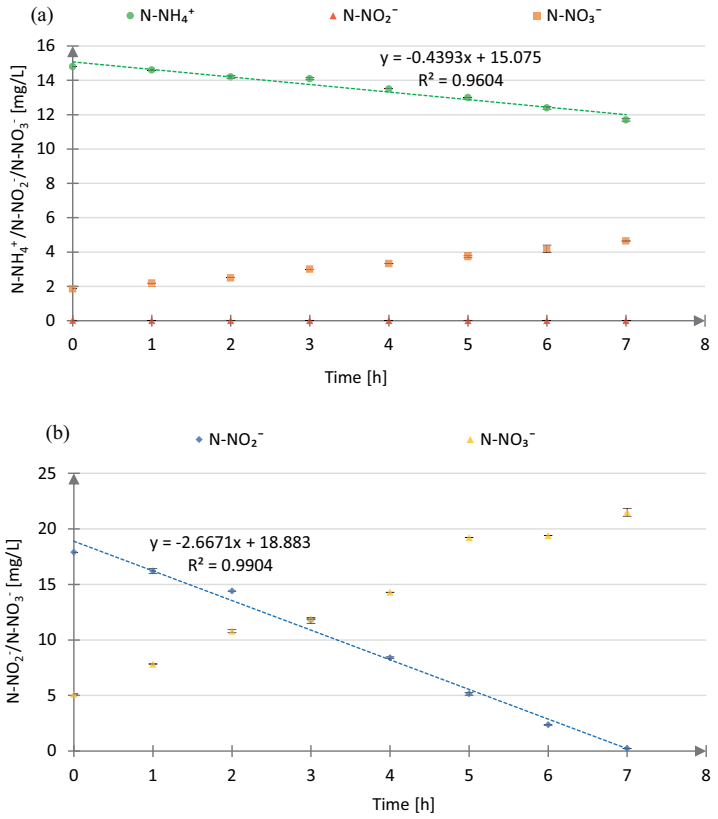

Fig. S9. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-B and (b) NitUR-B. 1st repetition – 12°C.

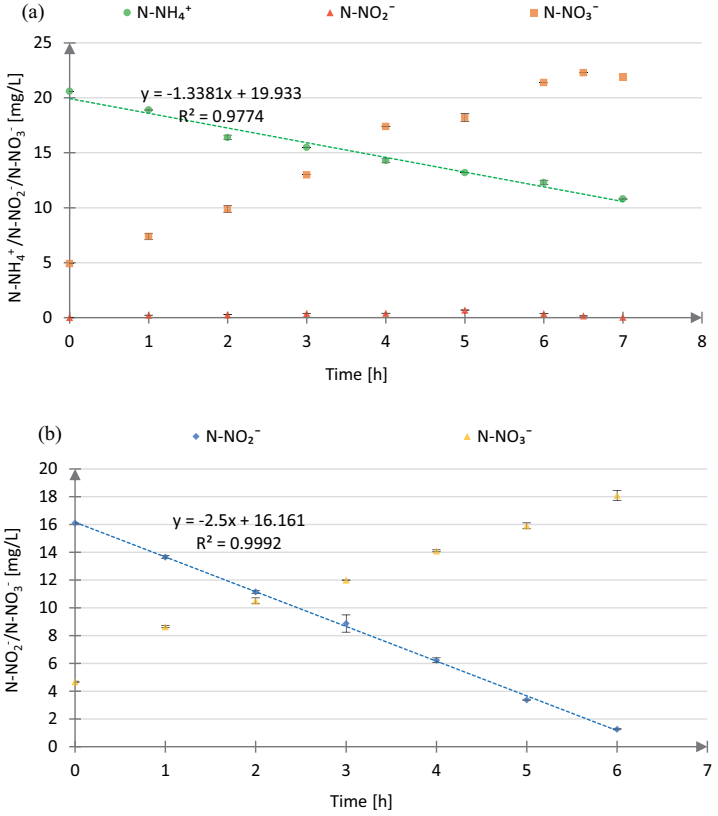

Fig. S10. N–NH_4^+, N–NO_2^-, N–NO_3^- profiles during test (a) AUR-H and (b) NitUR-H. 2nd repetition – 12°C.

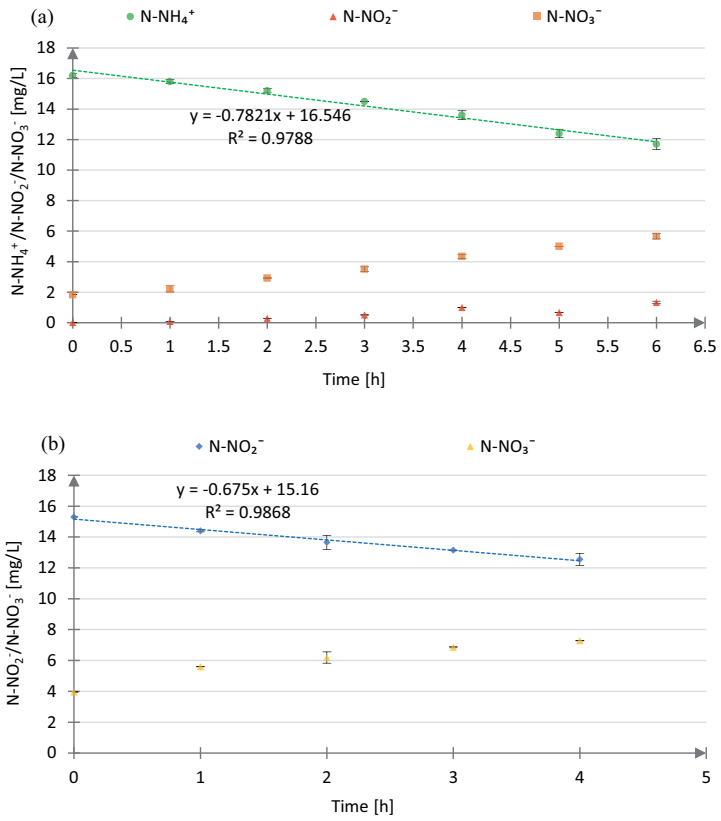

Fig. S11. $N–NH_4^+$, $N–NO_2^-$, $N–NO_3^-$ profiles during test (a) AUR-AS and (b) NitUR-AS. 2nd repetition – 12°C.

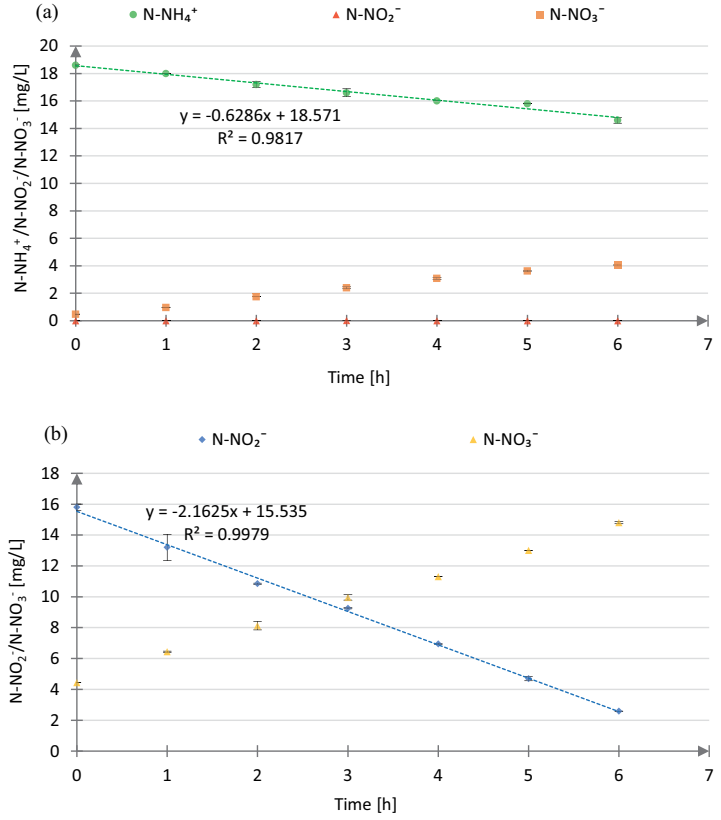

Fig. S12. $N–NH_4^+$, $N–NO_2^-$, $N–NO_3^-$ profiles during test (a) AUR-B and (b) NitUR-B. 2nd repetition – 12°C.