Substrate and Product Inhibition of Nitrification

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Great effort is paid to the development of new technologies, that enable the short-cut of the nitrification-denitrification cycle avoiding the oxidation of NO_2^- to NO_3^- (nitrite route). The nitrification of NH_4^+ only to NO_2^- and its reduction to gaseous N_2 offers several advantages: lower oxygen demand for nitrification, lower demand of organic matter for the denitrification of NO_2^-, higher denitrification rates of NO_2^-

The aim of this work was to obtain more detailed information about the inhibitive forms (dissociated or nondissociated) of the substrate/product as well as about the concentrations that cause inhibition. The substrate—product inhibition was tested in batch tests. Each experiment at a certain pH was carried out using a set of six reactors. One of them served as a reference, while the five others contained different concentrations of the tested compounds. Five sludges from different municipal wastewater treatment plants were used in the experiments. The results obtained from batch inhibition tests carried out on different sludges are briefly summarized in this work. The very similar behaviour of different sludges is worth to note.

One of the most common technologies to remove nitrogen from wastewaters is biological nitrification-denitrification. Nitrification is a process, in which NH_4^+ is gradually oxidized to NO_2^- (nitritation) and subsequently to NO_3^- (nitratation) by nitrifying microorganisms. The produced NO_3^- are then, under anoxic conditions, reduced to gaseous N_2 by heterotrophic microorganisms, utilizing the organic matter content of the wastewater. Regardless of some inhibitory influences, that can make difficult the use of these biological processes, even the lack of organic matter for denitrification can often have a negative impact on the overall nitrogen removal efficiency. In most of the cases, additional dosage of biodegradable organic matter (methanol, acetic acid, organic wastes, *etc.*) is used to resolve these problems. However, this additional dosage can increase the costs of the treatment process. Thus great effort is paid to the development of new technologies, that enable the short-cut of the nitrification-denitrification cycle avoiding the oxidation of NO_2^- to NO_3^- (the so-called nitrite route). The nitrification of NH_4^+ only to NO_2^- and its reduction to gaseous N_2 offers several advantages

– lower oxygen demand for nitrification (only 75 % of the amount necessary for complete nitrification to NO_3^-),

– lower demand of organic matter for the denitrification of NO_2^- (only 60 % of the demand necessary to the denitrification of NO_3^-),

– 40 % higher denitrification rates of NO_2^- [1],

– lower biomass yield during anoxic growth [2],

– possible adaptation of heterotrophic microorganisms to high concentrations of NO_2^- (up to $\rho(NO_2^-$—N) = 2000 mg dm^{-3} at pH 8—8.5) [1].

An interesting possibility to achieve only partial nitrification of NH_4^+ to NO_2^-, is the SHARON process [3]. It is based on the kinetic selection of ammonium oxidizers in a system without sludge retention. Another possibility to perform the nitrite route is based on the different sensibility of nitrifiers to the substrate—product inhibition [4], where nitrite oxidizers should be selectively more inhibited at lower NH_3 concentrations than ammonium oxidizers. Also, temperatures higher than about 25 °C are reported to favour nitrite accumulation [5].

The aim of this work was to obtain more detailed information about the inhibitive forms (dissociated or nondissociated) of the substrate/product as well as about the concentrations that cause inhibition. The obtained results should be utilized during the following research directed towards the short-cut of nitrification-denitrification. Despite of possible troubles caused by adaptation of the nitrite oxidizers to the substrate—product inhibition [2, 6], there could be cases, where specific conditions (high NH_4^+ and NO_2^- content of the wastewater connected with high or low pH, high salinity, specific inhibitors strengthening the substrate—product inhibition) can enable the long-term performance of the nitrite route.

EXPERIMENTAL

The substrate—product inhibition (in sequel as inhibition) was tested in batch tests lasting for 1—1.5 h. Each experiment at a certain pH was carried out using a set of six reactors ($V = 200\ cm^3$). One of them served as a reference, while the five others contained different concentrations of the tested compounds. The required concentrations of PO_4^{3-}, NH_4^+, NO_2^-, and NO_3^- were achieved by addition of K_2HPO_4, $(NH_4)_2SO_4$, $NaNO_2$, and $NaNO_3$ solutions. The pH was continuously controlled and maintained by addition of KOH and H_2SO_4 solutions. Five different sludges were used in the experiments:

- sludge A: a sludge coming from a nitrifying municipal wastewater treatment plant (MWWTP) with pure oxygen aeration (*ca.* 500 000 inhabitants),
- sludge B: a sludge coming from a MWWTP with nitrification, denitrification, and phosphorus removal in side stream (*ca.* 200 000 inhabitants),
- sludge C: a sludge coming from a nitrifying-denitrifying lab-scale sequencing batch reactor,
- sludge D: a sludge coming from a nitrifying MWWTP (*ca.* 150 000 inhabitants),
- in Fig. 2 even some results from [7] are plotted for comparison. These results were obtained with a sludge (sludge E) coming from a nitrifying MWWTP (*ca.* 350 000 inhabitants).

The concentrations of PO_4^{3-}—P and dissolved oxygen in the reactors were 3—5 mg dm^{-3} and 4—7 mg dm^{-3}, respectively. The concentrations of the activated sludge were in the range of 1—5 g dm^{-3}.

All analyses were carried out according to the Standard Methods [8], except the determination of NO_2^-, which was carried out using the Zambelli method [9] (a colorimetric method with sulfanilic acid and phenol).

RESULTS AND DISCUSSION

The concentrations of nondissociated NH_3, HNO_2, and HNO_3 were calculated on the basis of acid-base balances [4, 10]. The inhibition was evaluated using the equations of noncompetitive inhibition

$$I = \frac{\rho}{\rho + K_i} \qquad (1)$$

$$I = \frac{\rho - \rho_0}{\rho - \rho_0 + K_i'} \qquad (2)$$

where: I – inhibition, K_i, K_i' – inhibition constant (concentration causing an inhibition of 50 %), ρ_0 – concentration over which inhibition starts to demonstrate itself, ρ – concentration.

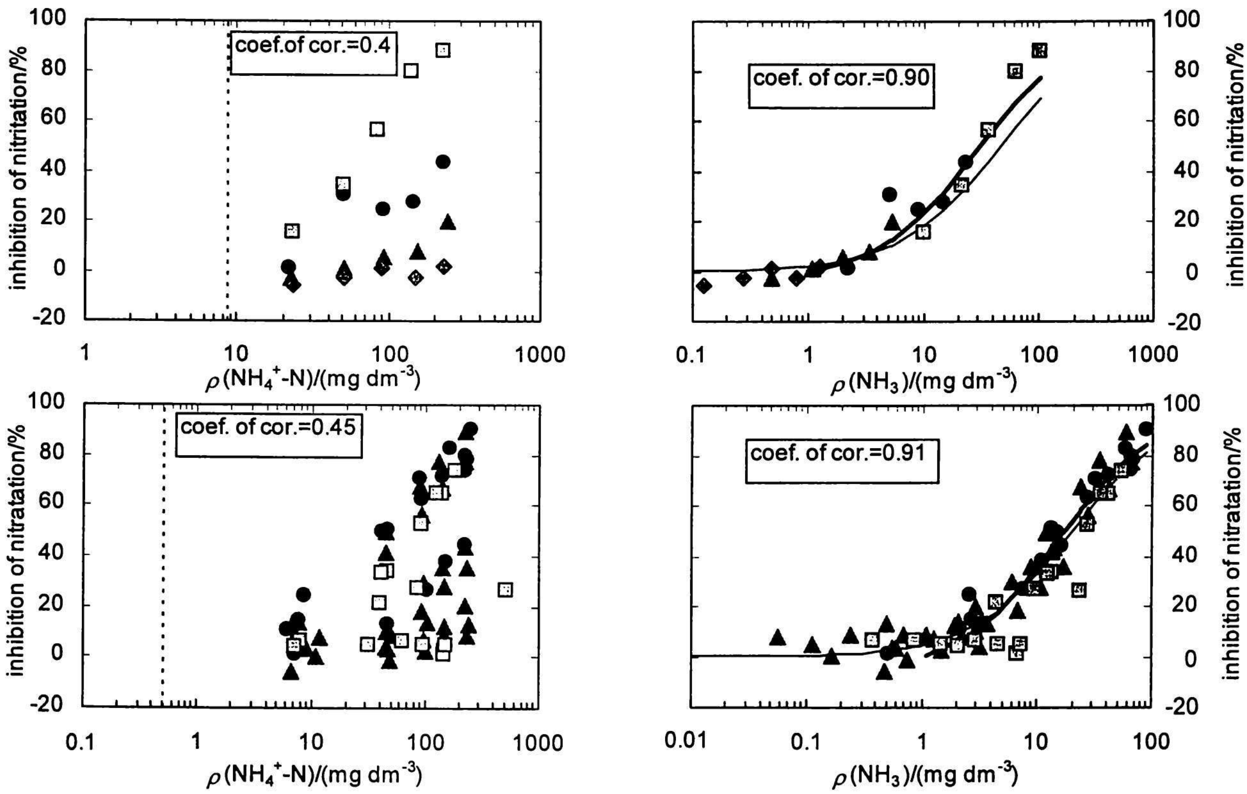

Fig. 1. Inhibition of nitrification by NH_4^+—N/NH_3 (inhibition of nitritation measured using sludge D, ■ pH = 8.9, ● pH = 8.2, ▲ pH = 7.5, ♦ pH = 6.8; inhibition of nitratation measured using sludges ● A, ▲ B, ■ C; —— $I = \rho/(\rho + K_i)$, —— $I = (\rho - \rho_0)/(\rho - \rho_0 + K_i')$).

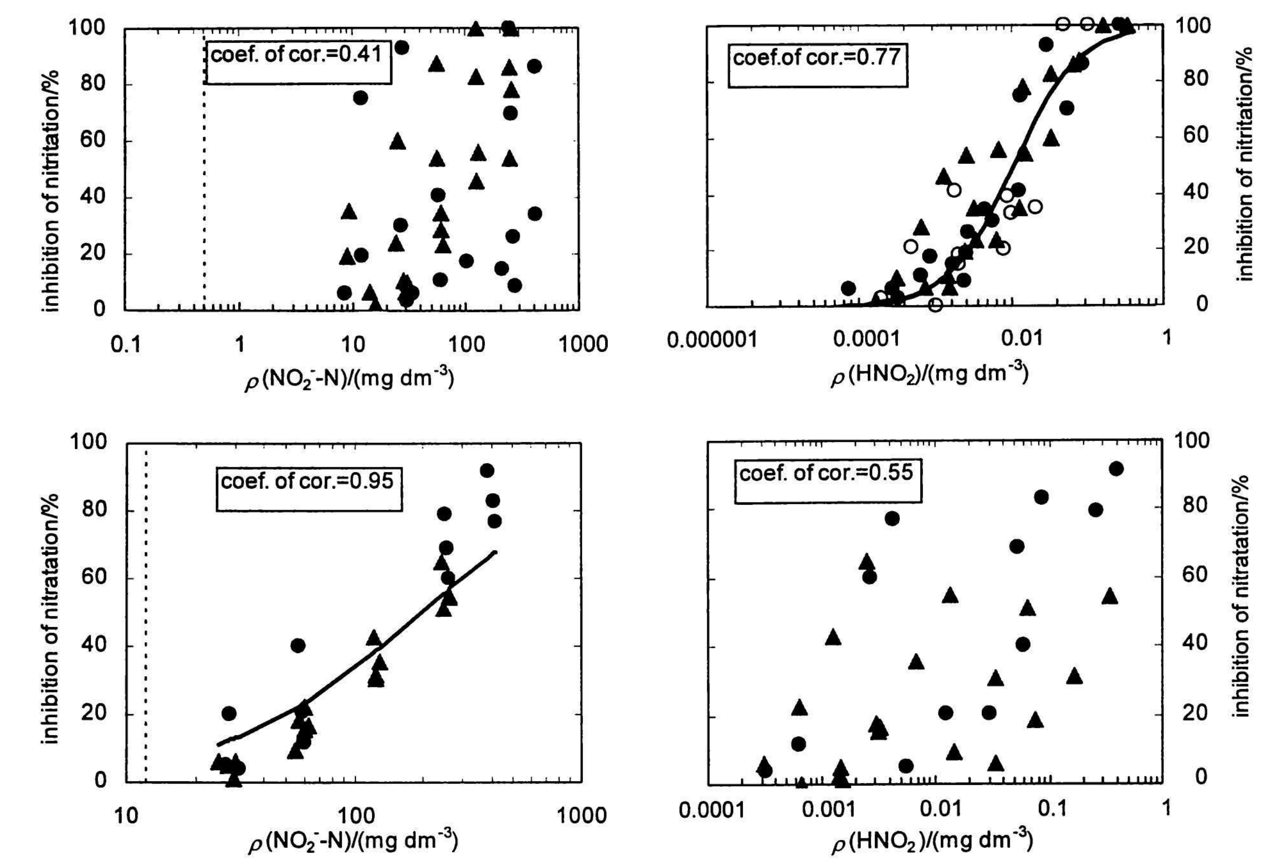

Fig. 2. Inhibition of nitrification by NO_2^-—N/HNO_2 (▲ sludge A, ● sludge B, ○ sludge E; —— $I = \rho/(\rho + K_i)$, —— $I = (\rho - \rho_0)/(\rho - \rho_0 + K_i')$).

Eqn (2) was used in cases, where there was clearly found that up to a certain concentration no inhibition occurred (inhibition occurred only at concentrations exceeding ρ_0 – see Fig. 1). The inhibition was calculated using the following equation

$$I = \frac{r_{ref} - r}{r_{ref}} \, 100 \ \% \qquad (3)$$

where: I – inhibition, r – nitritation/nitratation rate, r_{ref} – nitritation/nitratation rate in the reference reactor.

Correlation coefficients were calculated according to [11] as

$$\text{coef. of cor.} = \frac{n\sum xy - \sum x \sum y}{\sqrt{\left[n\sum x^2 - \left(\sum x\right)^2\right] \cdot \left[n \sum y^2 - \left(\sum y\right)^2\right]}} \qquad (4)$$

where: n – number of measurements, x – concentration of the inhibitive substance, and y – inhibition.

Inhibition of Nitrification by NH_4^+/NH_3

The tested ranges of pH and NH_4^+—N concentrations were 6.8—9 and 0—250 mg dm^{-3}, respectively. The reference reactors contained at the beginning of the experiments $\rho(NH_4^+—N)$ = 1 mg dm^{-3} and $\rho(NO_2^-—N)$ = 15 mg dm^{-3} (reference reactor for the nitratation), and $\rho(NH_4^+—N)$ = 13 mg dm^{-3} (reference reactor for the nitritation). The average concentration of the examined substance in the reference reactor is signed with a dashed line (in all of the figures). It is obvious from Fig. 1 (the horizontal axes of all of the figures are in logarithmic scale) that the inhibition of nitritation and nitratation was caused by the nondissociated form, the NH_3.

This becomes evident, as soon as the NH_4^+—N concentrations are converted to the NH_3 ones (see the coefficient of correlation). As the inhibition started only over a certain concentration (S_0), both equations of noncompetitive inhibition were utilized to describe it (both equations were utilized even for the HNO_2 and HNO_3 inhibition). The very similar behaviour of three different sludges is worth to note.

Inhibition of Nitrification by NO_2^-/HNO_2

The tested ranges of pH and NO_2^-—N concentrations were 6.8—8.9 and 0—415 mg dm^{-3}, respectively. The reference reactors contained at the beginning of the experiments $\rho(NH_4^+—N)$ = 15 mg dm^{-3} and $\rho(NO_2^-—N)$ = 15 mg dm^{-3} (reference reactor for the nitratation), and $\rho(NH_4^+—N)$ = 15 mg dm^{-3} (ref-

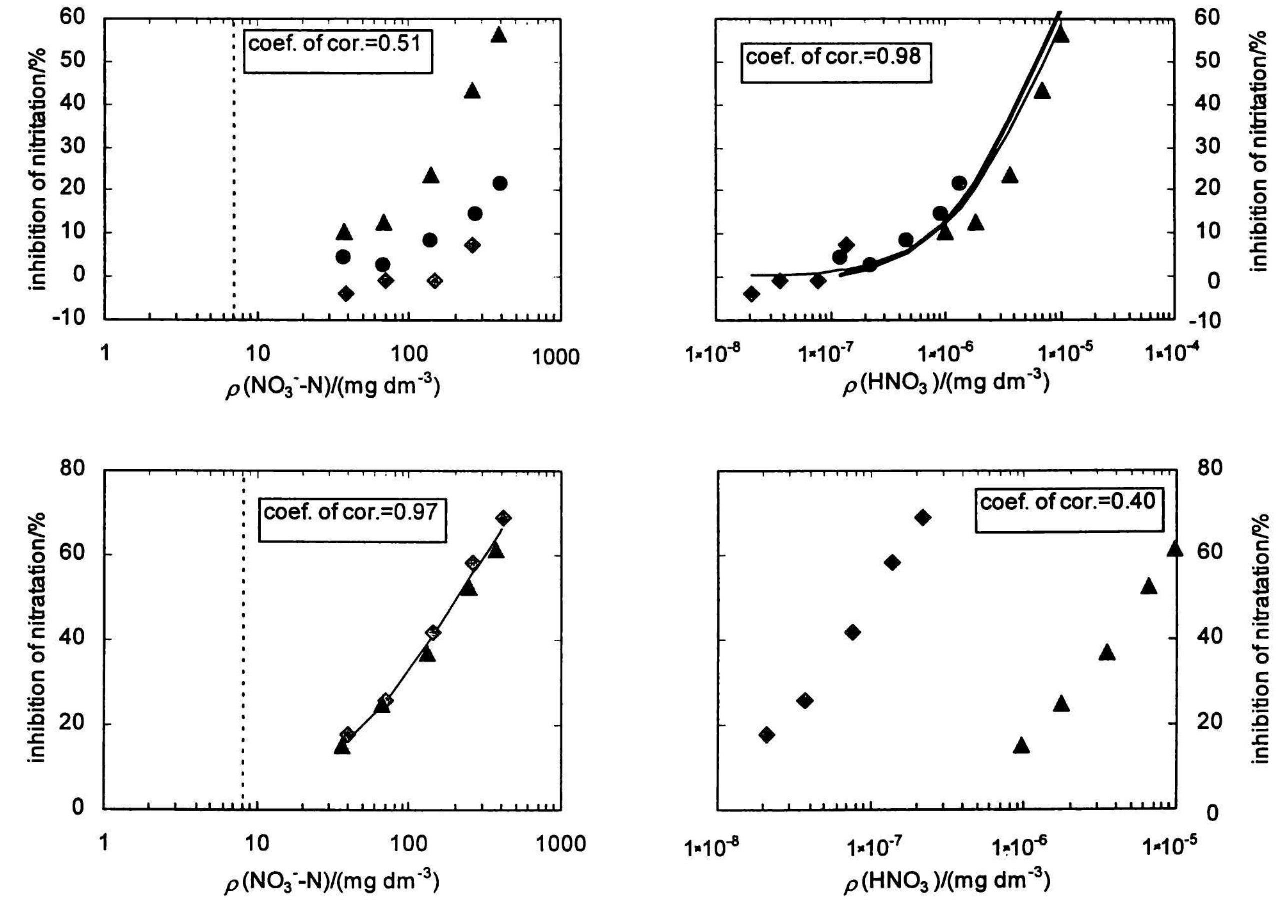

Fig. 3. Inhibition of nitrification by NO_3^-—N/HNO_3 (inhibition measured using sludge D, ◆ pH = 8.5, ● pH = 7.7, ▲ pH = 6.8; —— $I = \rho/(\rho + K_i)$, —— $I = (\rho - \rho_0)/(\rho - \rho_0 + K_i')$).

Table 1. Forms and Concentrations of NH_4^+/NH_3, NO_2^-/HNO_2, and NO_3^-/HNO_3 Causing Inhibition of Nitrification

Form causing inhibition	NH_4^+	NH_3	NO_2^-	HNO_2	NO_3^-	HNO_3
Nitritation	no	yes	no	yes	no	yes
Nitratation	no	yes	yes	no	yes	no

Concentration causing inhibition		NH_3	NO_2^-—N	HNO_2	NO_3^-—N	HNO_3
Nitritation	beginning of the inhibition (ρ_0/(mg dm^{-3}))	1	–	1×10^{-4}	–	8×10^{-8}
	inhibition of 50 % (K_i – eqn (*1*))/(mg dm^{-3})	46		0.0109	–	7×10^{-6}
	inhibition of 50 % (K_i' – eqn (*2*))/(mg dm^{-3})	30	–	0.0105	–	6×10^{-6}
Nitratation	beginning of the inhibition (ρ_0/(mg dm^{-3}))	1	–	–	–	
	inhibition of 50 % (K_i – eqn (*1*))/(mg dm^{-3})	20	198	–	205	–
	inhibition of 50 % (K_i' – eqn (*2*))/(mg dm^{-3})	16	–	–	–	–

Note: In cases of inhibition by dissociated forms it was not possible to determine ρ_0 from the obtained results.

erence reactor for the nitritation). As it is obvious from Fig. 2, the inhibition of nitritation was caused by the nondissociated HNO_2. The behaviour of different sludges, like previously in the case of NH_3 inhibition, was again quite uniform. Contrary to the commonly accepted theory of HNO_2 inhibition of nitratation, the dissociated NO_2^- was found to cause the inhibition of nitrite oxidizers. The inhibitive effect of a certain concentration of NO_2^- was not significantly strengthened by lowering the pH.

Inhibition of Nitrification by NO_3^-/HNO_3

The tested ranges of pH and NO_3^-—N concentrations were 6.8—8.5 and 0—400 mg dm^{-3}, respectively. The reference reactors contained at the beginning of the experiments $\rho(NH_4^+—N)$ = 1 mg dm^{-3} and $\rho(NO_2^-—N)$ = 15 mg dm^{-3} (reference reactor for the nitratation), and $\rho(NH_4^+—N)$ = 15 mg dm^{-3} (reference reactor for the nitritation). The nondissociated HNO_3 was found to cause the inhibition of nitritation (see Fig. 3), while the nitratation was inhibited by the dissociated NO_3^-

CONCLUSION

The nondissociated NH_3, HNO_2, and HNO_3 were found as inhibitive forms for the nitritation. The nondissociated HNO_2 and HNO_3 were expected to inhibit the nitratation [4], but on the basis of our results the nondissociated NH_3 and the dissociated NO_2^- and NO_3^- were found to inhibit the nitratation. Inhibitive concentrations of substrate/product, as well as the inhibition constants are summarized in Table 1. Comparing the inhibition constants, nitratation seems to be more sensitive to NH_3 inhibition than nitritation. All these experiments were carried out as batch tests, so the behaviour of the nitrifying microorganisms in long-term experiments can differ due to acclimatization. The substrate—product inhibition will be studied further in long-term experiments.

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