Nitrogen removal from wastewater by heterotrophic denitrification with nitrite used as an electron acceptor

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Abstract. The objective of the research was to determine the best conditions for heterotrophic denitrification by using nitrite as an electron acceptor. The results of the experiments indicate that heterotrophic denitrification where nitrite is used as the electron acceptor is practicable and feasible. However, the concentration of nitrite in the anoxic zone of the wastewater treatment plant has to be monitored constantly, as the accumulation of nitrite could cause a decrease in the denitrification activity. The process of full denitrification could be inhibited, if the concentration of NO_2^--N rose over 10–35 mg/L, depending on the operating conditions of the system.

Key words: denitrification, nitrite, activated sludge.

INTRODUCTION

For the welfare of natural water bodies receiving treated wastewater the removal of nutrients from wastewater has received much attention and become mandatory in recent years. The most commonly used process for nitrogen removal is biological nitrification–denitrification, carried out mainly by autotrophic and heterotrophic bacteria, respectively. Nitrification is carried out in two steps: oxidation of ammonium to nitrite (Eq. 1)

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O, \tag{1}$$
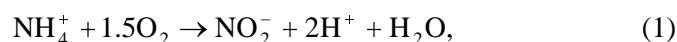
followed by nitrite oxidation to nitrate (Eq. 2)

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-. \tag{2}$$

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The main cost in the process is caused by aeration [1], which is obligatory to satisfy the need for oxygen necessary for the oxidation processes. As technical possibilities have enlarged, the economic feasibility, especially operational expenses of the facility, has become a major factor to be considered in designing the purification system. Partial nitrification is a novel approach in the biological nitrogen removal process, where the formulation of nitrate is terminated with the formation of nitrite (Eq. 1). The main advantage of partial nitrification instead of conventional, complete nitrification, is reduced oxygen demand, which gives considerable savings in aeration costs [2].

Denitrification is a process where oxidized nitrogen compounds are transformed into harmless nitrogen gas by heterotrophic bacteria. Reduction of nitrite instead of nitrate results in a lower requirement for organic substrate, which is obligatory for heterotrophic denitrification [3, 4]. This constitutes another economic advantage in the cases where wastewater does not contain enough easily degradable organic matter and an external organic substrate, for example methanol, has to be added to avoid incomplete denitrification, i.e. emission of N_2O [5, 6].

High nitrite concentration, which may be achieved through partial nitrification, can reduce the activity of microorganisms responsible for the purification process in general. Nitrite concentrations higher than 8 mg NO_2^--N/L can already inhibit the biological removal of phosphorus [7]. According to several authors [8, 9], NO_2^--N concentrations starting from 10–30 mg/L will decrease the activity of biomass, including facultative anaerobic bacteria, contriving full denitrification.

A factor that reduces denitrification efficiency, independently from nitrite, is dissolved oxygen (DO) [10, 11] because most of the common denitrifying bacteria are facultative anaerobes, preferring oxygen to nitrite as the electron acceptor. Even if the concentration of DO is as low as 0.1 mg/L, noticeable inhibition of denitrification could appear [12]. A low value of mixed liquor suspended solids (MLSS) in the anoxic tank of a real-scale wastewater treatment plant (WWTP) can create conditions where the concentration of DO is not always zero. The presence of DO reduces the efficiency of denitrification, creating the possibility that nitrite will be present in the effluent of WWTP. The discharge of nitrite into natural water bodies has to be avoided, since it is toxic to biomass [13, 14]. Therefore, when heterotrophic denitrification is used for nitrogen removal from nitrite as the electron acceptor, the influence of two major factors – DO and NO_2^- – has to be considered for the process effectiveness.

MATERIALS AND METHODS

In the experiments activated sludge from three different municipal WWTPs was used. Operational characteristics of the selected WWTPs are described in Table 1.

Table 1. Operational characteristics of WWTPs providing activated sludge

Abbreviation of WWTP and sludge in text	WWTP loading, kg BOD_7/d	Water flow rate, m^3/d	N_{tot} in influent, mg/L	Nitrogen removal obligatory	Concentration of DO in aeration tank, mg/L
A	6 740	21 000	57	Yes	1–2
B	149	2 485	70	No	$0.5 < DO < 1.5$
C	600	2 757	51	Since 2004	$0.5 < DO < 1.5$

All the chosen WWTPs were based on activated sludge technology. According to the regulations valid, nitrogen removal from wastewater is currently obligatory in WWTP A. WWTP C will have to meet these requirements from the year 2004.

Experimental setup

Two types of laboratory scale reactors, with a volume of 500 mL, were used for the experiments. One type was equipped with a pH electrode, a dissolved oxygen control unit, and an additional stirrer. The other type was equipped with a pH electrode and a stirrer. The reactor was sealed with an air-tight cap and equipped with an "oxygen free" sample taking system, making the presence of a DO sensor dispensable. All reactors were filled with activated sludge and synthetic wastewater containing acetate, substantial nutrients, and nitrite nitrogen. The experiments were carried out at nitrite concentrations ranging from 0.5 to 80 mg/L. The concentration of the biomass was kept equal to 1 g MLSS/L through all the experiments.

To distinguish the precise influence caused by DO, half of the test solutions was deoxygenated with inert gas argon before start-up. The other half of the experiments was carried out in reactors where a detectable amount of DO was present. During the experiments the pH of the test solution was maintained between 7 and 7.4. For this Na_2CO_3 was added if necessary.

Temperature was through all the experiments kept at 20 °C, which is below the optimum level for the denitrification process, as in the real size WWTP temperature would be a rather complicated and the most expensive parameter to regulate for the optimal denitrification process.

Analytical methods

As nitrite can inhibit the process of denitrification, special attention was paid to the determination of process dependence on nitrite concentration. The influence of DO was studied taking into account other factors that could affect denitrification. Temperature was monitored and considered as a side condition, affecting mainly the velocity of the process.

For optimal elucidation of process dependences the duration of every single experiment was four hours. Samples from test solutions were taken before the

start up and during the experiment after every hour. For a better kinetic overview, sampling was done from selected experiments after every half an hour.

The indicators measured from the samples were the concentration of NH_4^+, NO_2^-, NO_3^-, and DO, and pH. Ammonium and nitrate were analysed using ion selective electrodes (Jenway) and colorimetrically, in accordance with the Finnish standard method SFS 3032 [15] and ISO 7890-3 standard [16], respectively. Nitrite concentration was measured colorimetrically, following the Finnish standard method SFS 3029 [17]. The concentration of dissolved oxygen was measured with an oxygen meter (Marvet Junior) and pH with a pH meter (Evikon).

RESULTS AND DISCUSSION

NO_2^--N influence

As the effectiveness of denitrification is tightly related to the initial concentration of nitrite, the aim of the current research was to determine the maximum concentration of NO_2^--N that would not inhibit denitrification and also the optimum concentration of nitrite corresponding to the highest initial velocity for the denitrification process. Experiments were done in anoxic conditions. Possible side effects caused by DO were minimized by special pre-treatment of the test solutions and complete isolation from the oxygenated environment.

Figures 1 and 2 give an overview of the changes of nitrite concentrations through the experiment in the case of sludges A and B, respectively. On the basis of measured nitrite concentrations denitrification velocities were calculated. The initial velocity of denitrification for a given amount of nitrite, corresponding to the maximum rate of nitrite removal, was applied to assess the studied processes.
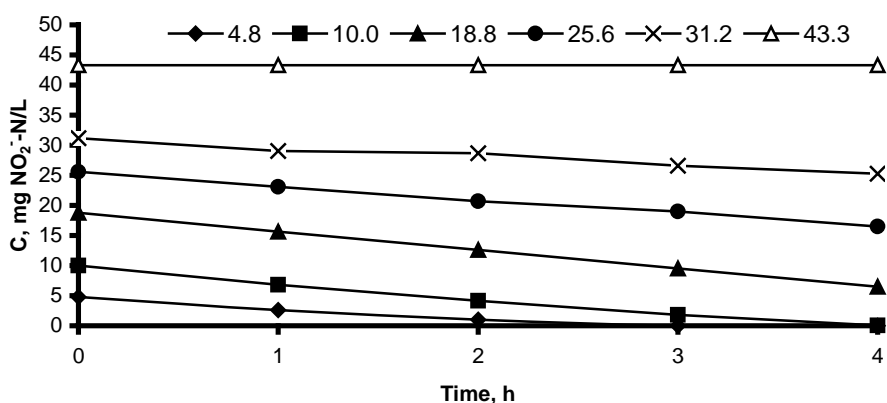

Fig. 1. Sludge A, reduction of nitrite on diverse initial NO_2^--N concentrations.

In the case of sludge A the highest concentration of NO_2^--N allowing complete denitrification in four hours was 10.0 mg/L. The maximum removal of nitrite was achieved at a concentration of 18.8 mg NO_2^--N/L, with the removal effectiveness of 66%. However, this was already in the region of nitrite concentration where denitrification was slightly influenced by the inhibiting effect of the substrate, as it was seen from the initial velocity of the process. At higher concentrations of nitrite the rate of denitrification started to decrease progressively due to the inhibition caused by the substrate. The rate of denitrification reached the zero level at a concentration of 43.3 mg NO_2^--N/L. At nitrite concentrations higher than 18.8 mg/L a major decrease in the initial velocity of the process was observed. In the case of lower nitrite concentrations the velocity of denitrification decreased in time due to the decrease of the nitrite concentration related to the reduction of nitrite.

The highest efficiency of nitrite removal was achieved by using sludge B. Complete denitrification was reached at a concentration of 16.5 mg/L NO_2^--N. The maximum rate of nitrite removal was at 26.7 mg NO_2^--N/L. When the nitrite concentration was increased the efficiency of denitrification fell, resulting in 19% of total nitrite removal at a concentration of 83.5 mg NO_2^--N/L. As shown in Fig. 2, even at a concentration of 65.5 mg NO_2^--N/L denitrification was achievable, although the initial velocity was lower than at smaller concentrations and the process velocity continued to decrease through the experiment.

Compared to the tests with sludges A and B, sludge C showed moderate process parameters. The nitrite removal rate was the highest at a concentration of 20.1 mg/NO_2^--N/L and the removal efficiency of nitrite was 73% (during 4 h). Complete denitrification was achieved at 9.8 mg NO_2^--N/L. At concentrations higher than 50 mg/L the denitrification process stopped completely. The maximum initial velocity of nitrite removal with sludge C was 5.3 mg NO_2^--N/h. This parameter for sludge A and B was 3.2 and 8.1 mg NO_2^--N/h, respectively.
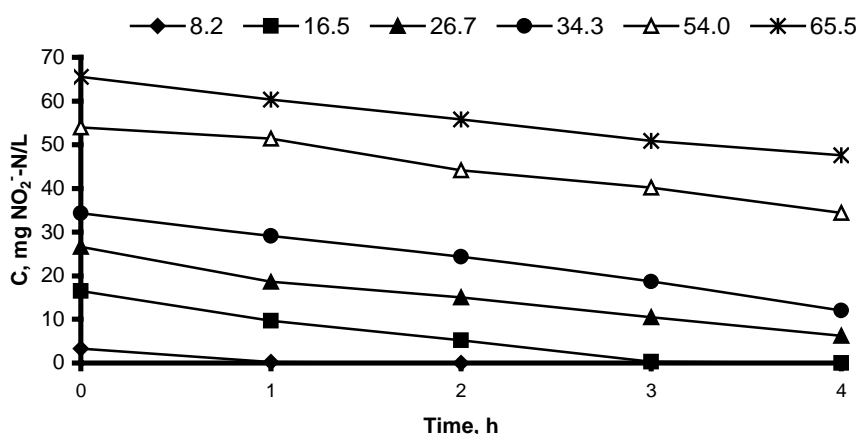

Fig. 2. Sludge B, reduction of nitrite on diverse initial NO_2^--N concentrations.

Still, despite of the lowest initial velocity, sludge C indicated quite stable process effectiveness.

In order to predict the theoretical performance for the denitrification process by activated sludge mathematical modelling was carried out. Taking into consideration that the kinetics of denitrification is based on enzyme activity, the rate equation describing the process by activated sludge can be based on the Michaelis–Menten equation for the two substrate process. During the denitrification the nitrite is consumed as one of the substrates by microorganisms. In the experiments the organic substrate was used in excess, so that it can be considered as a constant and therefore the denitrification initial velocity can be expressed as follows:

$$v_0 = v_{MAX} \frac{S_{NO_2^-}}{K_{s_{NO_2^-}} + S_{NO_2^-}}, \tag{3}$$

where v_{MAX} is the maximum rate of the process, $S_{NO_2^-}$ is the concentration of nitrite, and $K_{s_{NO_2^-}}$ is the half-saturation constant for nitrite.

Initial velocities of the process for different nitrite concentrations in the experiment were calculated, and further the dependence of the initial velocities on the nitrate concentration was investigated, as shown in Figs. 3 and 4.

The shape of the experimental curves in Figs. 3 and 4 indicates that the Michaelis–Menten model does not fit to describe the process at high concentrations of nitrite. The deviation of the obtained dependences from the hyperbolic function at high nitrite concentrations can probably be explained by the inhibition effect of nitrite, as observed also by several authors [8, 9]. Assuming an inhibiting effect of nitrite, one of the substrates of the process, we tried to describe the obtained dependences by Haldane's equation [18, p. 126]:

$$v_0 = v_{MAX} \frac{S_{NO_2^-}}{K_{s_{NO_2^-}} + S_{NO_2^-} + \dfrac{S_{NO_2^-}^2}{K_i}}, \tag{4}$$

where K_i is the inhibition constant.

Equation 4 is applicable in the case of substrate inhibition, but it did not fit to describe the obtained dependences of the initial velocity of denitrification on nitrite concentration. Therefore, modification of Haldane's equation was carried out. Finally Eq. 5 was found to be appropriate to characterize the studied process:

$$v_0 = v_{MAX} \frac{S_{NO_2^-}}{K_{s_{NO_2^-}} + S_{NO_2^-} + \dfrac{S_{NO_2^-}^2}{K_i} + K_{ii} S_{NO_2^-}^3}, \tag{5}$$

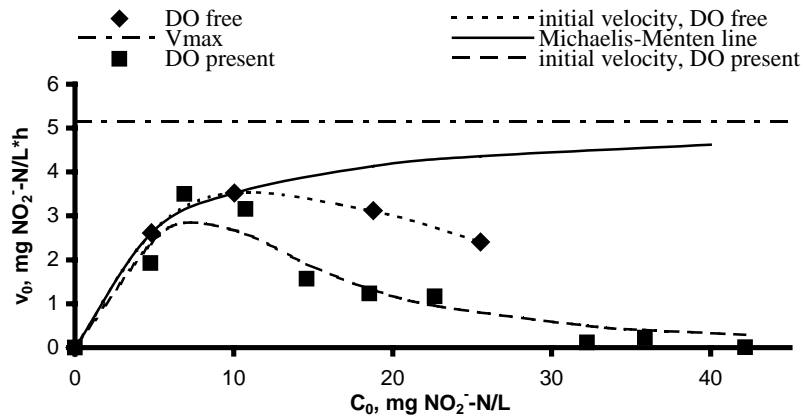where K_i and K_{ii} are inhibition constants.

Fig. 3. Sludge A, the dependence of denitrification initial velocity on different nitrite concentrations and dissolved oxygen.
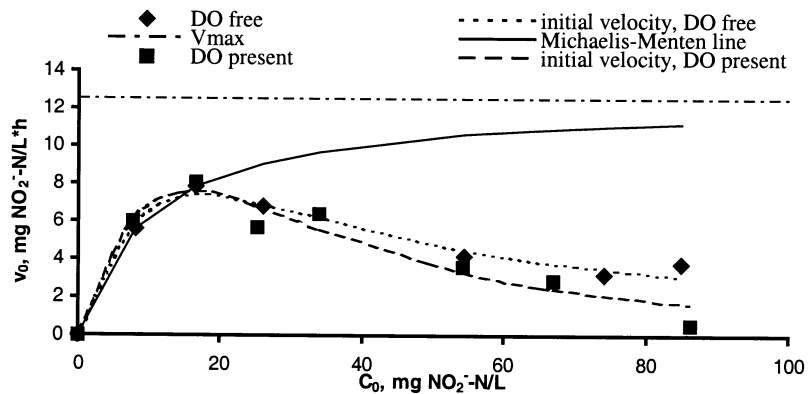

Fig. 4. Sludge B, the dependence of denitrification initial velocity on different nitrite concentrations and dissolved oxygen.

Figures 3 and 4 illustrate the experimentally measured data along with the theoretically predicted lines obtained on the basis the above-mentioned model equation by using non-linear regression and the method of the least squares.

DO influence

To predict the consequences of aerated water inflow to the anoxic zone or oxygen diffusion through the water surface, denitrification tests at DO concentrations between 0.1 and 0.7 mg/L were also done. To evaluate possible negative impacts of DO, all the experiments were accomplished in parallel with direct NO_2^--N inhibition tests done in DO free conditions.

The experimental results showed that up to the concentration of 0.2 mg/L, DO has no clearly visible effect on the denitrification process. Most of the experiments indicated that the DO concentrations higher than 0.2 mg/L explicitly reduced the toleration of bacteria to NO_2^--N. Table 2 gives an overview about the experimentally measured NO_2^--N concentrations that were found to be optimal considering the highest initial velocity of the denitrification process.

The results presented in Table 2 vary in quite a wide range, which indicates that the parameters characterizing the biological denitrification process depend on the environmental conditions under which the microorganisms were kept before the experiments. The repressive effect of DO appears more clearly with increasing nitrite concentration as DO amplifies the decrease in the efficiency of the denitrification process caused by nitrite.

The major difference compared with DO free conditions is in general the abatement of initial velocities of the process, leading to a decrease in optimal nitrite concentrations. Taking into consideration different results, the optimal concentration of nitrite for denitrification in aerated environment is up to 43% lower.

Figure 3 shows initial velocities of denitrification at different concentrations of nitrite when sludge A was used. As mentioned above, denitrification efficiency begins to decrease if the concentration of nitrite rises over 10.7 mg/L in the oxygenated environment. In oxygen free conditions the initial velocity is substantially higher and achievable at higher nitrite concentrations.

In the case of sludge B the maximum initial velocity of denitrification was not essentially influenced by the presence of oxygen (Fig. 4). However, in the oxygenated environment the rise of the process velocity was notably more abrupt than in oxygen free conditions. This cannot be explained by further oxidation of nitrite to nitrate or reduction of nitrite to ammonium, as during the experiments the measured nitrite and ammonium concentrations remained almost constant. As shown in Table 2, the optimal concentration of NO_2^--N for satisfactory denitrification in the case of sludge B in aerated conditions was 16.5 mg/L, although the inhibition of denitrification started already at the NO_2^--N concentration of 14 mg/L.

Sludge C demonstrated utmost dependence on DO as can be seen in Table 2. The difference in the initial velocities of the processes between oxygenated and oxygen free conditions was up to two times.

Table 2. Optimal NO_2^--N concentrations for denitrification

DO, mg/L	Sludge A*		Sludge B*		Sludge C*	
	Opt NO_2^--N, mg/L	v, $\dfrac{\text{mg } NO_2^-\text{-N}}{h}$	Opt NO_2^--N, mg/L	v, $\dfrac{\text{mg } NO_2^-\text{-N}}{h}$	Opt NO_2^--N, mg/L	v, $\dfrac{\text{mg } NO_2^-\text{-N}}{h}$
$0.2 < DO < 0.7$	10.7	3.1	16.5	8.1	23.7	2.8
$DO = 0$	18.8	3.2	26.7	8.1	20.1	5.3

* See Table 1 for characteristics

In general, as shown in Figs. 3 and 4, the presence of DO in wastewater-activated sludge suspensions can cause a decrease in the initial velocity of the denitrification process. Knowledge of the initial velocity of the process allows us to predict the effectiveness of the whole denitrification process.

CONCLUSIONS

In this research heterotrophic denitrification was used for the elimination of nitrite produced from wastewater by partial nitrification. In the anoxic reactor the concentration of nitrite has to be constantly monitored because accumulation of nitrite would cause a decrease in denitrification activity. The results of this research indicated that the effectiveness of full denitrification would be markedly reduced if the concentration of NO_2^--N in the denitrification system rose over 18–27 mg/L. The process rate also depends on the operating conditions of the system. If denitrification, due to technical reasons, has to be carried out in the environment with dissolved oxygen concentration up to 0.7 mg/L, the highest concentration of NO_2^--N tolerable to biomass is 10–24 mg/L.

It was shown that heterotrophic denitrifying organisms in municipal wastewater can use nitrite as an electron acceptor. The described process offers additional savings thanks to lower biochemical oxygen demand and provides effective nitrogen removal, especially if the C/N ratio in wastewater is naturally low. However, it is necessary to consider that for successful and reliable denitrification, the concentration of nitrite in the anoxic reactor has to be monitored during the process and, if necessary, adjusted periodically.

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Reovees leiduva lämmastiku ärastamine, kasutades denitrifikatsioonil elektronaktseptorina nitritit

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On uuritud osalise nitrifikatsiooniprotsessi korral tekkiva nitriti denitrifikatsiooni võimalusi. Selgus, et nitriti akumulatsioon võib põhjustada denitrifikatsiooni aktiivsuse olulist vähenemist. Eksperimentide tulemustest ilmnes, et sõltuvalt aktiivmudast väheneb denitrifikatsiooni efektiivsus oluliselt NO_2^--N kontsentratsioonil 18–27 mg/l. Kui denitrifikatsioon toimub tingimustes, kus vees lahustunud hapniku kontsentratsioon ulatub kuni 0,7 mg/l, siis aktiivmuda omadustest sõltuvalt langeb biomassi poolt talutav nitritlämmastiku kontsentratsioon tasemeni 10–24 mg/l.

Võttes kokku eksperimendi tulemusi, võib öelda, et heterotroofsed denitrifitseerivad organismid on võimelised kasutama nitritlämmastikku denitrifikatsioonil elektronaktseptorina. Protsessi rakendamisega kaasneva kergesti lagundatava orgaanilise aine vajaduse vähenemine annab märkimisväärse majanduskasu. Siiski peab nitritil põhineva denitrifikatsiooni rakendamisel võtma pideva vaatluse alla NO_2^--N kui protsessi võimaliku inhibiitori kontsentratsiooni muutumise, et tagada protsessi efektiivsus. Vajadusel tuleb süsteemis leiduva nitritlämmastiku sisaldust korrigeerida.