

# Inhibition of Encapsulated Nitrite Oxidizing Bacteria by Short-time Exposure to Hydroxylamine

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## Abstract

Partial nitrification brings oxygen demand of 25% lower than conventional nitrification. Next advantage can be lower chemical oxygen demand (COD) consumption during following denitrification which is appropriate for wastewater with high  $N_{\text{amon}}$  concentration and low COD/N ratio.

Nitration inhibition can become because of higher concentration of nitrification substrates, products and intermediates: ammonium, nitrite, nitrate and hydroxylamine ( $\text{NH}_2\text{OH}$ ). When suspended biomass is used, full nitration inhibition starts at  $\text{N-NH}_2\text{OH}$  concentration of 0.42 mg/l. It is recommended to use  $\text{N-NH}_2\text{OH}$  concentration between 0.15 and 2.33 mg/l. In the case of biomass immobilised on biofilters  $\text{N-NH}_2\text{OH}$  concentration of 2.5-5.0 mg/l is needed.

Immobilisation of biomass supports slowly growing microorganisms such as nitrification bacteria. One of immobilisation methods is an encapsulation which covers microorganisms in some stable material. Encapsulated biomass is protected against external conditions, is less sensitive on inhibitors presence, pH value or temperature changes. Technology LentiKat's uses encapsulated biomass in PVA carriers which have a shape of lens.

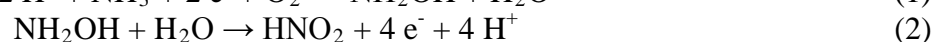
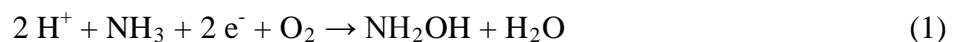
## Keywords

Encapsulation; hydroxylamine; immobilisation; LentiKat's; partial nitrification

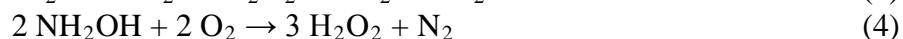
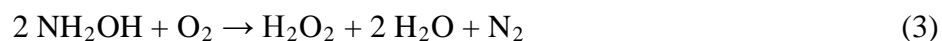
## INTRODUCTION

The most common method of nitrogen removal is biological treatment called nitrification. Nitrification consists of two processes: nitritation and nitration. During nitritation ammonia nitrogen ( $N_{\text{amon}}$ ) is oxidised to nitrite nitrogen ( $\text{N-NO}_2$ ) due to AOB (Ammonia-Oxidizing Bacteria). Then NOB (Nitrite-Oxidizing Bacteria) oxidise nitrite nitrogen to nitrate one. Partial nitrification brings oxygen demand of 25% lower. Next advantage can be decreased chemical oxygen demand (COD) consumption which is appropriate for wastewater with high  $N_{\text{amon}}$  concentration and low COD/N ratio.

Nitration inhibition can become because of higher concentration of nitrification substrates, products and intermediates: ammonium, nitrite, nitrate and hydroxylamine ( $\text{NH}_2\text{OH}$ ). One of the theories mentions hydroxylamine as the reason of nitration inhibition with high ammonium concentration and low oxygen concentration. In the system which is not aerated enough, hydroxylamine could be accumulated according to equations 1 and 2.

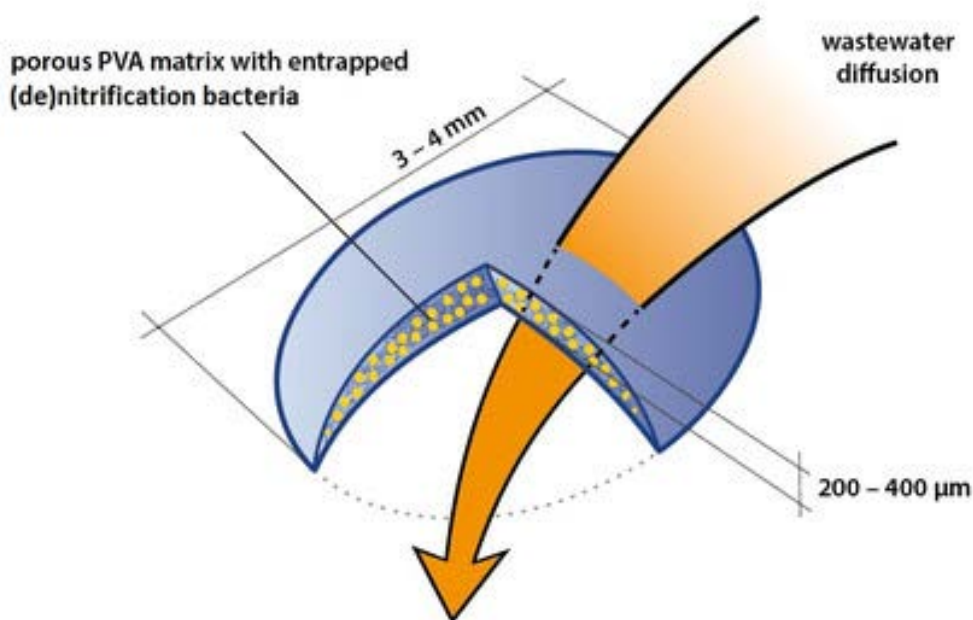


Another theory explains nitration inhibition with hydroxylamine become because of his reaction with gas oxygen when hydrogen peroxide is made according to equations 3 and 4.



When suspended biomass is used, full nitration inhibition starts at N-NH<sub>2</sub>OH concentration of 0.42 mg/l. It is recommended to use N-NH<sub>2</sub>OH concentration between 0.15 and 2.33 mg/l. In the case of biomass immobilised on biofilters N-NH<sub>2</sub>OH concentration of 2.5-5.0 mg/l is needed.

Immobilisation of biomass supports slowly growing microorganisms such as nitrification bacteria. One of immobilisation methods is an encapsulation which covers microorganisms in some stable material, e.g. polyvinylalcohol (PVA) or polyethylenglykol (PEG). Encapsulated biomass is protected against external conditions and is less sensitive on inhibitors presence, pH value or temperature changes. Technology LentiKat's uses encapsulated biomass PVA carriers which have a shape of lens (**Fig. 1**).



**Figure 1.** Scheme of the LentiKat's pellet

## MATERIALS AND METHODS

### Hydroxylamine dose

The aim of this study was to test an impact of hydroxylamine concentration on encapsulated nitrification bacteria. In our laboratory, reactor (**Fig. 2**) was filled with 1 l diluted sludge water (**Tab. 1**) and 50 g of LentiKat's pellets (*Nitrosomonas europaea*, *Nitrobacter winogradskyi*). This sequencing batch reactor worked with retention time of 3.3 days. Dissolved oxygen concentration was maintaining at higher value than 4 mg/l. During 11 hours kinetic test pH value was 7.0-7.3. At the beginning of the kinetics tests NH<sub>2</sub>OH.HCl was applied (initial N-NH<sub>2</sub>OH concentration of 10-200 mg/l).

**Table 1.** Average constitution of diluted sludge water

Nitrogen form/COD	Concentration [mg/l]
N <sub>amon</sub>	516
N-NO <sub>2</sub>	0.2
N-NO <sub>3</sub>	1.4
COD	1258



**Figure 2.** Reactors used for experiments of nitration inhibition with hydroxylamine

### Hydraulic retention time

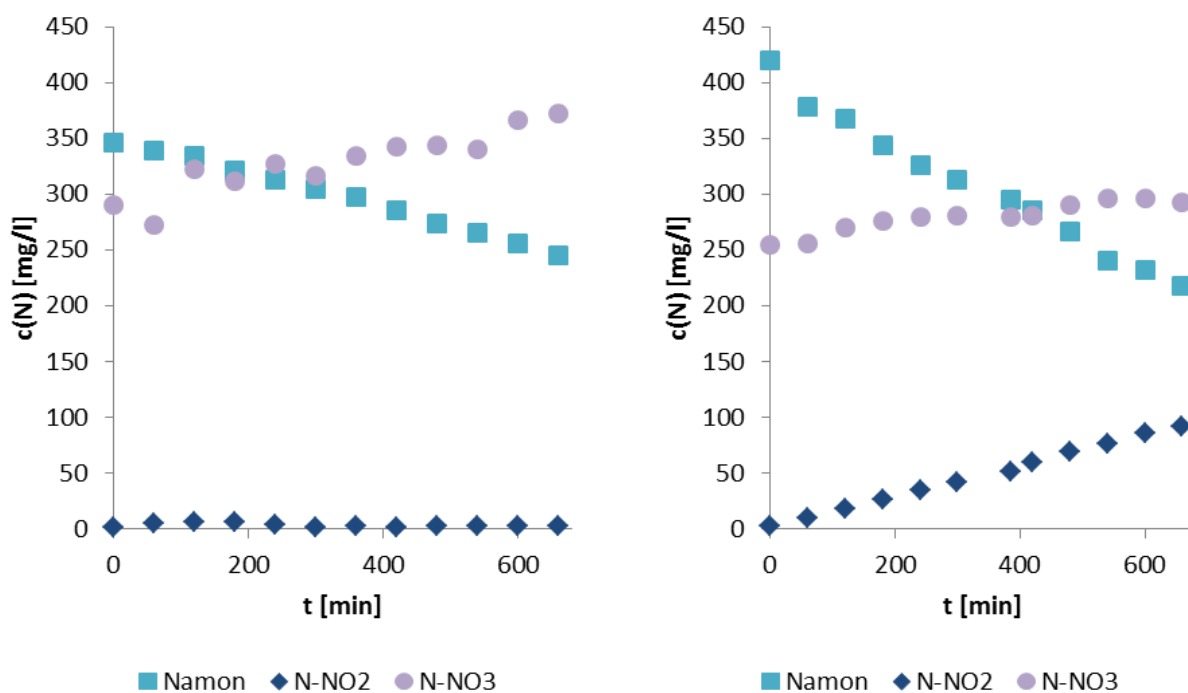
The aim of this test was to find out an impact of hydraulic retention time (HRT) on nitration inhibition with hydroxylamine. In our laboratory, reactor was filled with 1.2 l diluted sludge water and 100 g of LentiKat's pellets (*Nitrosomonas europaea*, *Nitrobacter winogradskyi*). N<sub>amon</sub> loading was treated on value of 200 mg/(l.d) and pH value of  $7,2 \pm 0.15$ . N-NH<sub>2</sub>OH concentration added at the beginning of the kinetic test was 200 mg/l. 1-day and 3-days HRT were tested.

### RESULTS AND DISCUSSION

**Fig. 3** shows the difference between kinetics test with initial N-NH<sub>2</sub>OH concentration of 0 and 50 mg/l. Higher nitrite accumulation and lower nitrate production are visible. When N-NH<sub>2</sub>OH of concentration of 50 mg/l was dosed, inhibition of nitration was achieved for more than one week; however the activity of NOB was restored thereafter.

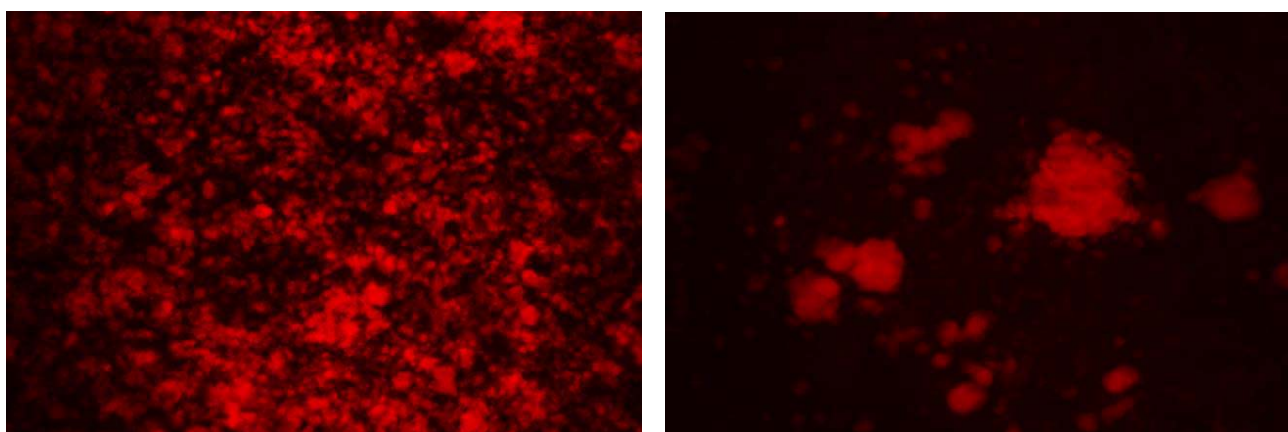
It should be mentioned that hydroxylamine makes a positive error during N<sub>amon</sub> analysis. That is the reason for higher initial N<sub>amon</sub> concentration when hydroxylamine was present.

In the tests of HRT impact higher N-NO<sub>2</sub> accumulation was found for HRT of 3 days. While in the case of 1-day HRT maximal N-NO<sub>2</sub> concentration was 150 mg/l, when 3-days HRT was used N-NO<sub>2</sub> concentration of 500 mg/l was measured.



**Figure 3.** Influence of N-NH<sub>2</sub>OH 0 mg/l (left) and 50 mg/l (right) on N<sub>amon</sub>, N-NO<sub>2</sub> and N-NO<sub>3</sub> concentration

Interesting findings were found also during fluorescence *in situ* hybridization (FISH). In **Fig. 4** can be seen the comparison of AOB before the tests and after hydroxylamine addition. It was found that when bacteria in the pellets are stressed they are make clusters.



**Figure 3.** Comparison of AOB before (left) and after (right) hydroxylamine addition

## CONCLUSION

Unfortunately, the N-NH<sub>2</sub>OH concentration needed for full nitrification inhibition has not been found. However, partial nitrification with immobilised biomass using nitrification inhibition with hydroxylamine could be an appropriate method for nitrogen removal from water with high N<sub>amon</sub> concentration. Because of higher chemical resistance of bacteria which are immobilised in PVA pellets the N-NH<sub>2</sub>OH concentration needed is higher than in the case of suspended biomass.

## ACKNOWLEDGEMENTS

Financial support from Ministry of industry and trade of the Czech Republic (project FR -TI4/254) and from specific university research (MSMT No 20-SVV/2016).

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