

Nitrogen Oxidation and Reduction in Aerated Single-Stage Activated Sludge Process

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Abstract

This experiment was conducted in an aerated single-stage reactor at activated sludge concentration of about $3000 \text{ mg} \cdot \text{dm}^{-3}$. The influence of hydraulic retention time on nitrogen removal from rural wastewater was investigated. Hydraulic retention times were 24 and 12 hours. Two series were performed at a constant C/N ratio of 3.5. Enhancement of carbon removal from 83.8% to 87.6% was observed. An increase of activated sludge loading from $0.11 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ to $0.25 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ did not cause total inhibition of nitrification, but nitrification efficiency decreased from 78.8% to 55.5%. Higher nitrogen removal efficiency (52%) was achieved in series I at 24 hours reaction time in contrast to shorter one (42%).

Despite the fully aerobic condition, denitrification was observed. The denitrification process was possible due to the use of accessibility of high amount of readily bioassimilable organic compounds. Obtained denitrification efficiency at C/N ratio 3.5 was about 50% during both experimental periods.

Keywords: nitrification rate, aerobic denitrification, nitrogen removal, biomass synthesis, activated sludge

Introduction

Conversions of nitrogen compounds in aerated conditions carried out by pure cultures are widely reported in the literature [1, 2, 3]. Van Niel, et al. [4] described growth and denitrification under fully aerobic conditions through facultatively autotrophic bacterium *Thiosphaera pantotropha*. The authors showed that the enzymes involved in heterotrophic nitrification are in many respects similar to those found in the chemolithotrophic ammonia-oxidizing bacteria. *Thiosphaera pantotropha* is able to oxidize ammonium by means of enzymes typically present in nitrifying bacteria *Nitrosomonas*. Robertson, et al. [3] proved that at dissolved oxygen concentrations of

30 to 80% of air saturation *Thiosphaera pantotropha* can use simultaneously both nitrate and oxygen as electron acceptors. Thus, it is the aerobic denitrifier that possesses in its cells constitutive denitrifying enzymes. In the studies of Robertson et al. [3] nitrite accumulation was not observed. If nitrite accumulation occurred it would mean an inhibition of the first step of nitrification or the second phase of denitrification. Nitrite only accumulated in medium during aerobic conditions when substances limiting denitrification additionally inhibited nitrate reductases.

Patureau et al. [2] reported that *Alcaligenes faecalis*, *Pseudomonas nautica*, *Comamonas sp.* and *Microvirgula aerodenitrificans* [5] are capable of denitrifying under fully aerobic conditions. During initial research the cultures of pure nitrifying strain were used. After stabilization of the nitrifying activity, 100% of ammonia oxidation to ni-

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trate was observed. In the following phase pure aerobic denitrifiers and additional carbon sources were supplied to the medium. As for strain *Comamonas sp.* products of ammonia oxidation were not observed.

Immediately after the addition of the pure strain of *Microvirgula aerodenitrificans* and acetic acid, the N-oxidized products were reduced. The obtained results confirmed that under fully aerobic conditions ammonium was nitrified by autotrophic microflora, heterotrophic aerobic denitrifiers simultaneously consumed the N-oxides.

The existence, growth and possibility of aerobic denitrification by *Citrobacter diversus* were reported by Huang, et al. [1]. With regard to the effect of oxygen most aerobic denitrifiers discovered appear to have denitrification activity when the dissolved oxygen concentration is below $3 \text{ mg} \cdot \text{dm}^{-3}$. *Citrobacter diversus* may tolerate a dissolved oxygen concentration up to $5 \text{ mg} \cdot \text{dm}^{-3}$.

Only some research has confirmed the possibility of nitrogen removal from wastewater under aerobic conditions by mixed population of activated sludge. Effective nitrification by the heterotrophic bacteria was found in activated sludge, where most of the microorganisms were heterotrophic. The result of ammonium conversion to gaseous nitrogen carried out by the same groups of bacteria comes from the fact that aerobic denitrifiers can be heterotrophic nitrifiers, too [3, 6]. The oxidation of an inorganic source of reductant coupled to N-oxide reduction is sometimes termed autotrophic denitrification.

The studies of Zhao, et al. [7] showed that heterotrophic ammonia oxidation is relevant and occurs when the C/N ratio is low and the organic carbon loading of activated sludge is high. Under these conditions 15 to 50% reduction of N-oxide forms is achieved.

Simultaneous nitrification and denitrification in activated sludge was observed [8] when dissolved oxygen concentration was about $5.6 \text{ mg} \cdot \text{dm}^{-3}$. The results of Simpkin, et al. [9] indicated that there was no repression of denitrifying enzymes synthesis in activated sludge. The induction of nitrate/nitrite reductases under aerobic conditions was at least 50%

of their maximum possible activity levels.

There is a lack of results in literature concerning technological research on nitrogen removal from wastewater by mixed cultures of activated sludge under aerobic conditions. Our experiment was carried out in a single-stage reactor with the activated sludge when activated sludge loading changed. The aim of the research was the documentation of nitrogen oxidation and reduction under constant oxygen supplying to the reactor that assure oxygen concentration on the level of $2 \text{ mg} \cdot \text{dm}^{-3}$.

The range of research included the determination of the ammonification, nitrification and denitrification rates and making the nitrogen compounds balance in a one-stage activated sludge reactor under constant aeration.

Experimental Procedures

Single-Stage Reactor

The experiment was conducted in a sequencing batch reactor BIOFLO 3000 at the volume of 5 dm^3 . The constantly aerated tank was fitted out with the system of oxygen supply, stirrer, automatic oxygen, temperature and pH controller systems. The reactor was exploited at the activated sludge concentration of $3000 \text{ mg} \cdot \text{dm}^{-3}$ and constant level of oxygen supply so that during endogenous phase the amount of oxygen concentration was equal to 30% of oxygen saturation at 20°C . The cycle of sequencing reactor work is shown in Fig. 1.

Organisation of Experiment

Hydraulic retention time of the reaction was 12 or 24 h in dependence on the experimental series. Volumetric exchange rate of the reactor was stable at 50%. 2.5 dm^3 of rural wastewater was supplied to the sequencing batch reactor. Raw wastewater contained considerable amounts of ammonium and suspended solids (Tab. 1).

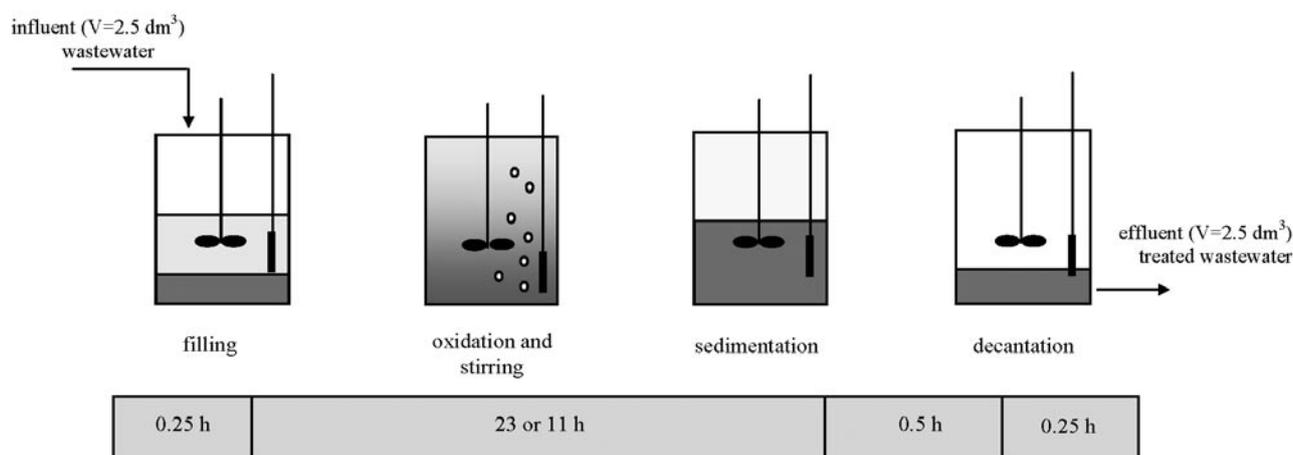


Fig. 1. Scheme of reactor SBR work.

Table 1. Chemical characteristics of wastewater.

Determination	Unit	Series I	Series II
COD (C_{COD})	$\text{mg O}_2 \times \text{dm}^{-3}$	577.8	650.3
Suspended solids	$\text{mg SS} \times \text{dm}^{-3}$	338.7	267.8
TKN	$\text{mg TKN} \times \text{dm}^{-3}$	135	141
N-NH_4	$\text{mg N-NH}_4 \times \text{dm}^{-3}$	101.9	111.8

In both series activated sludge was adapted to technological conditions. After the adaptation period changes of the chemical parameters in the influent and effluent were determined. The kinetic research was conducted at the end of each technological series while changes of oxygen, nitrogen compounds and organic compounds (COD) were analysed.

Analytical Methods

The following parameters were determined in the wastewater:

- organic compounds, expressed as total and dissolved COD [PN 74/C-04578/03] in the influent and effluent
- volatile fatty acids [PN 75/C-4616/04] in the influent and effluent
- ammonia by the Nesslerization method after distillation [PN 73/C-04576/01] or colorimetric method [PN 73/C-04576/02] in the influent and effluent
- total Kjeldahl nitrogen [PN 73/C-04576/12] in the influent and effluent
- total suspended solids by drying at 103-105°C [PN 73/C-04559/02] in the influent and effluent
- nitrite [PN 73/C-04576/06] and nitrate [PN 73/C-04576/08] by colorimetric methods in the effluent
- biomass concentration in the reactor by drying at 103-105°C [PN 73/C-04559/02].

Calculation Methods

Total ammonia amount that appeared during ammonification was calculated using the following formula:

$$\text{N-NH}_{4\text{amon}} = \text{N-NH}_{4\text{i}} + (\text{N}_{\text{orgi}} - \text{N}_{\text{orge}})$$

$\text{N-NH}_{4\text{amon}}$ - ammonium concentration obtained after ammonification ($\text{mg} \cdot \text{dm}^{-3}$),

$\text{N-NH}_{4\text{i}}$ - ammonium concentration in the influent ($\text{mg} \cdot \text{dm}^{-3}$),

N_{orgi} - organic nitrogen concentration in the influent [t = 0h] ($\text{mg} \cdot \text{dm}^{-3}$),

N_{orge} - organic nitrogen concentration in the effluent [t = 12 or t = 24h] ($\text{mg} \cdot \text{dm}^{-3}$).

Ammonification rate was calculated with the assumption that neither nitrification nor biomass synthesis occur in activated sludge.

The rate of ammonium loss was calculated on the basis of the difference between ammonia concentration that appeared during ammonification and ammonia concentra-

tion in the reactor at reaction time.

Total N-oxides during nitrification by activated sludge were conducted using the following formula:

$$\text{N-NO}_{\text{xnitr}} = \text{TKN}_i - (\text{N}_{\text{syn}} - \text{TKN}_e)$$

$\text{N-NO}_{\text{xnitr}}$ - concentration of nitrogen oxidized after nitrification ($\text{mg} \cdot \text{dm}^{-3}$),

TKN_i - concentration of total nitrogen in the influent [t = 0h] ($\text{mg} \cdot \text{dm}^{-3}$),

N_{syn} - concentration of nitrogen used for biomass synthesis ($\text{mg} \cdot \text{dm}^{-3}$),

TKN_e - concentration of total nitrogen in the effluent [t = 12 or t = 24h] ($\text{mg} \cdot \text{dm}^{-3}$).

Nitrification rate in activated sludge was estimated on the basis of nitrogen oxidized concentration changes during reaction time.

Amount of nitrogen reduced during denitrification was calculated using the following formula:

$$\text{N-NO}_{\text{xred}} = (\text{N-NO}_{\text{xnitr}} + \text{N-NO}_{2\text{e1}} + \text{N-NO}_{3\text{e1}}) - \text{N-NO}_{3\text{e2}} - \text{NNO}_{2\text{e2}}$$

$\text{N-NO}_{\text{xred}}$ - concentration of nitrogen reduced ($\text{mg} \cdot \text{dm}^{-3}$),

$\text{N-NO}_{2\text{e1}}$ - nitrite concentration in the reactor left from the previous day ($\text{mg} \cdot \text{dm}^{-3}$),

$\text{N-NO}_{3\text{e1}}$ - nitrate concentration in the reactor left from the previous day ($\text{mg} \cdot \text{dm}^{-3}$),

$\text{N-NO}_{2\text{e2}}$ - nitrite concentration in the effluent ($\text{mg} \cdot \text{dm}^{-3}$),

$\text{N-NO}_{3\text{e2}}$ - nitrate concentration in the effluent ($\text{mg} \cdot \text{dm}^{-3}$).

On the basis of N-reduced concentration changes in the reactor during reaction time denitrification rate was estimated.

Nitrification efficiency was calculated using the following formula:

$$\%N = \frac{\text{N-NO}_{\text{xnitr}}}{\text{TKN}_i} \cdot 100$$

Denitrification efficiency was calculated using the following formula:

$$\%DEN = \frac{\text{N-NO}_{\text{xred}}}{\text{N-NO}_{\text{xnitr}} + \text{N-NO}_{2\text{e1}} + \text{N-NO}_{3\text{e1}}} \cdot 100$$

Nitrogen removal efficiency was calculated using the following formula:

$$\%DEN = \frac{\text{N-NO}_{\text{xred}} + \text{N}_{\text{syn}}}{\text{TKN}_i + \text{N-NO}_{2\text{e1}} + \text{N-NO}_{3\text{e1}}} \cdot 100$$

Classification of COD (C_{COD}) in the influent on soluble and particulate fractions is used in designing and modelling of biological nutrient removal systems. In our experiment it was estimated on the basis of Barnard, et al. [10].

Concentration of the particulate fraction ($C_{\text{p,COD}}$) was calculated using the following formula:

$$C_{\text{p,COD}} = C_{\text{COD}} - C_{\text{s,COD}}$$

C_{COD} - organic carbon compounds concentration ($\text{mg} \cdot \text{dm}^{-3}$),

$C_{\text{s,COD}}$ - soluble fraction of organic carbon compounds concentration after filtration ($\text{mg} \cdot \text{dm}^{-3}$).

Concentration of slowly or non-biodegradable particulate fraction ($C_{\text{ps,COD}}$) was calculated using the following formula:

$$C_{\text{ps,COD}} = C_{\text{COD}} \cdot 0.05$$

0.05 - coefficient according to Barnard et al. [10].

Concentration of biodegradable particulate fraction ($C_{\text{pb,COD}}$) was calculated using the following formula:

$$C_{\text{pb,COD}} = C_{\text{p,COD}} - C_{\text{ps,COD}}$$

Concentration of slowly or non-biodegradable soluble fraction ($C_{\text{ss,COD}}$) was calculated using the following formula:

$$C_{\text{ss,COD}} = C_{\text{COD}} \cdot 0.03$$

0.03 - coefficient according to Barnard et al. [10].

Concentration of very readily biodegradable soluble fraction ($C_{\text{srb,COD}}$) was calculated using the following formula:

$$C_{\text{srb,COD}} = \frac{\text{VFA} \cdot 645}{1000}$$

VFA - volatile fatty acids concentration in wastewater (CH_3COOH), $\text{mg} \cdot \text{dm}^{-3}$

645 - 1 g/dm^3 of acetic acid correspond to 645 mg COD/dm^3 .

Concentration of readily biodegradable soluble fraction ($C_{\text{srb,COD}}$) was calculated using the following formula:

$$C_{\text{srb,COD}} = C_{\text{s,COD}} - C_{\text{svr,COD}} - C_{\text{ss,COD}}$$

Results

Concentration of organic compounds (C_{COD}) in raw rural wastewater in both series was similar. Soluble fraction of carbon compounds concentration ($C_{\text{s,COD}}$) was approximately 70% ($381.6 \text{ mg} \cdot \text{dm}^{-3}$ in series I and $449.3 \text{ mg} \cdot \text{dm}^{-3}$ in series II), however particulate fraction ($C_{\text{p,COD}}$) made up 30% of total organic carbon expressed as COD ($196.2 \text{ mg} \cdot \text{dm}^{-3}$ in series I and $201.2 \text{ mg} \cdot \text{dm}^{-3}$ in series II). Classification of carbon fractions in raw rural wastewater is shown in Table 2.

C/N ratio in the reactor at $t=0 \text{ h}$ in both series was about 3.5. Technological experiment in series I was carried out at the organic carbon loading of activated sludge of $0.11 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, in series II – $0.25 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$. The increase of organic carbon loading caused the increase of nitrogen compounds loading from $0.03 \text{ g N} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ to $0.07 \text{ g N} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$. Other technological parameters are shown in Table 3.

The constant amount of oxygen concentration that was supplied to the sequencing batch reactor with activated sludge, was equal to 30% of oxygen saturation at 20°C . Oxygen concentration curves indicate permanent complete oxygen use by activated sludge during initial reaction time despite the fact that oxygen was constantly delivered to the reactor. Total exhaustion of oxygen by activated sludge in series I during the period of time from $t=0 \text{ h}$ to $t=4 \text{ h}$ was observed. In series II the biomass was using constantly supplied oxygen during 5 initial hours of the experiment. After those times in both series increase of oxygen concentration to the amount of $2 \text{ mg} \cdot \text{dm}^{-3}$ in the reactor was observed (Fig. 2).

From Fig. 2 it may be concluded that during the initial reaction period oxidation processes and biomass synthesis predominated in activated sludge. In 24-hour series after 3 hours of reaction time 60% of dissolved carbon compounds, expressed as COD, was reduced. It made up about $110 \text{ mg COD} \cdot \text{dm}^{-3}$ and $70 \text{ mg COD} \cdot \text{dm}^{-3}$ of this quantity was used for biomass synthesis. In 12-hour series it is clear that for the first 2 hours of reaction time carbon removal efficiency was above 61%, which made up about $140 \text{ mg COD} \cdot \text{dm}^{-3}$. The use of these carbon compounds

Table 2. Contribution of the particular fraction of COD in wastewater.

Series		I	II
Soluble COD ($C_{\text{s,COD}}$) [$\text{mg} \times \text{dm}^{-3}$]	very readily biodegradable ($C_{\text{srb,COD}}$)	96.3	99.9
	readily biodegradable ($C_{\text{srb,COD}}$)	268	329.7
	slowly or non-biodegradable ($C_{\text{ss,COD}}$)	17.3	19.7
Particulate COD ($C_{\text{p,COD}}$) [$\text{mg} \times \text{dm}^{-3}$]	biodegradable ($C_{\text{pb,COD}}$)	167.3	168.7
	slowly or non-biodegradable ($C_{\text{ps,COD}}$)	28.9	32.5
C/N ratio in reactor		3.3	3.6

Table 3. Technological parameters of the series.

Determination	Unit	Series I	Series II
Hydraulic retention time	[h]	24	12
Sludge age	[d]	31.8	15.8
Yield of the biomass [Y]	[g × g ⁻¹]	0.62	0.79
Organic loading of activated sludge	[g × g ⁻¹ × d ⁻¹]	0.11	0.25
Nitrogen loading of activated sludge	[g × g ⁻¹ × d ⁻¹]	0.03	0.07

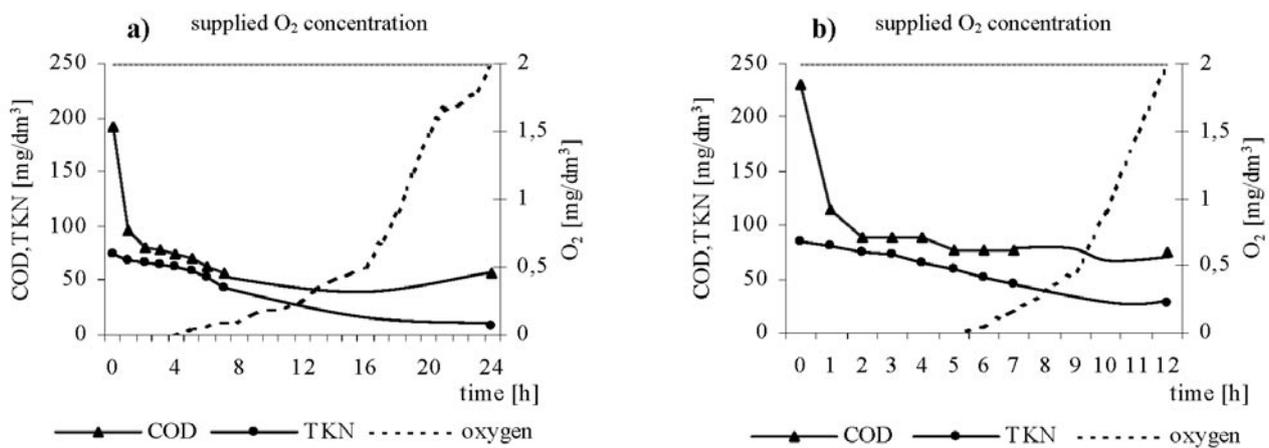


Fig. 2. Changes of dissolved oxygen concentration and curves of nitrogen and carbon removal by activated sludge under fully aerated condition in the reactor, a) series I, b) series II.

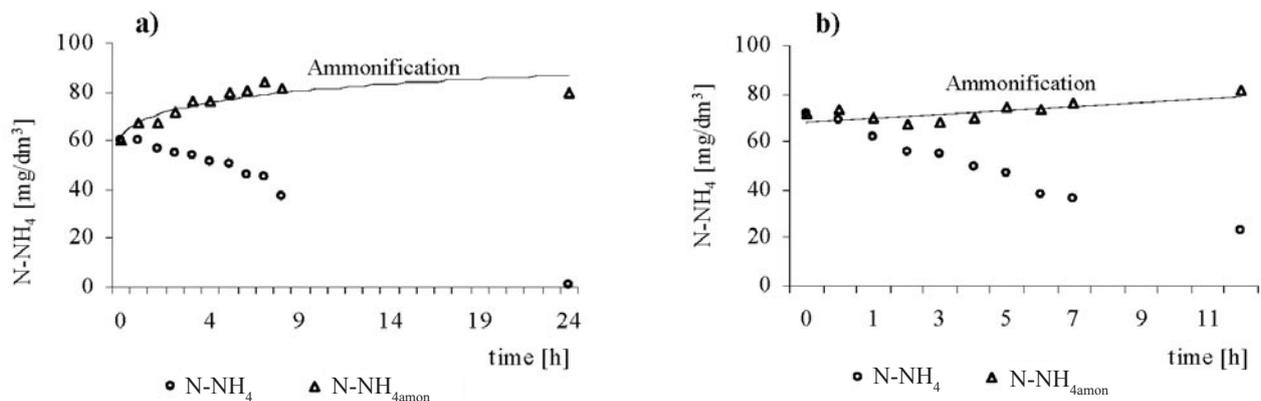


Fig. 3. Ammonification effect and ammonium loss in the reactor a) series I, b) series II.

for biomass synthesis was almost 80%. At the end, after 24 and 12 hours of the experiment period carbon removal efficiency was 83.8% in series I and 87.6% in series II.

Total nitrogen concentration in the reactor at a time $t=0$ h was 75 mg TKN · dm⁻³ in series I and 85 mg TKN · dm⁻³ in series II. In both series for the first three hours of the reaction time total nitrogen concentration decreased above 12% of the initial amount. Ammonium made up about 80% of TKN in wastewater in both series. For series I at

$t=0$ h ammonium concentration was 65 mg N-NH₄ · dm⁻³ and after 24 hours of reaction time it decreased to 0.66 mg N-NH₄ · dm⁻³. For 12-hour series the initial ammonium concentration was 72 mg N-NH₄ · dm⁻³ and ammonium consumption was observed to the amount of 22.8 mg N-NH₄ · dm⁻³. In either case ammonification rate was 0.35 mg N-NH₄ · g⁻¹ · h⁻¹. However, the ammonium loss rate for series I reached the value of 1.4 mg N-NH₄ · g⁻¹ · h⁻¹ but for series II was higher – 2.35 mg N-NH₄ · g⁻¹ · h⁻¹. Evolution

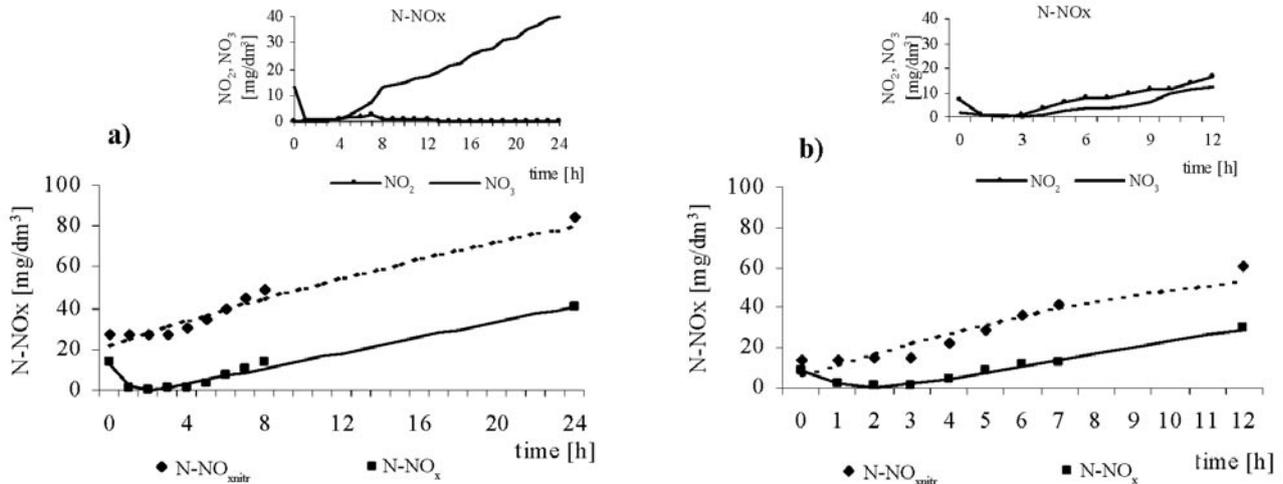


Fig. 4. Evolution of N-oxides after nitrification by activated sludge and nitrite and nitrate concentration in the reactor a) series I, b) series II.

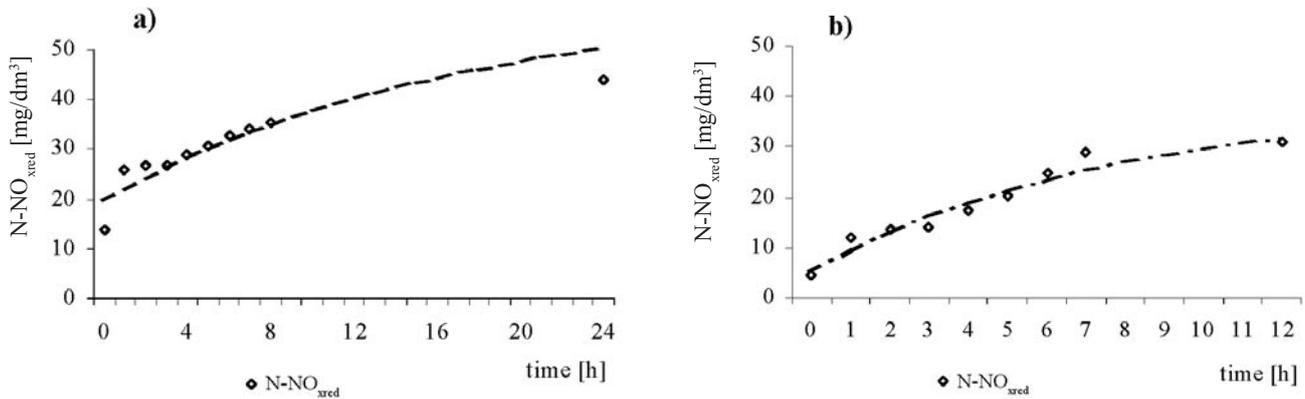


Fig. 5. Effect of nitrogen removal during denitrification a) series I, b) series II.

of ammonium concentration during ammonification and changes of ammonium concentration in activated sludge during reaction time are shown in Fig. 3.

Ammonium concentration used for the biomass synthesis reached higher value ($11.26 \text{ mg N-NH}_4 \cdot \text{dm}^{-3}$) for series II than for the 24-hour one ($8.13 \text{ mg N-NH}_4 \cdot \text{dm}^{-3}$).

Despite the fact that during the first initial hours of the reaction ammonium was accessible in wastewater in the reactor, an increase of N-oxidized forms was not observed. It is interesting that in both series the initial available quantity of nitrite and nitrate ($t = 0 \text{ h}$; series I - $13 \text{ mg NO}_2, \text{NO}_3 \cdot \text{dm}^{-3}$, series II - $9 \text{ mg NO}_2, \text{NO}_3 \cdot \text{dm}^{-3}$) was completely consumed by activated sludge. After only 3 hours in series I and after 4 hours in series II N-oxidized products started to appear. Fig. 4 shows the curve of ammonium oxidation forms by activated sludge and the sum of nitrite and nitrate present in the reactor during reaction time.

At the activated sludge loading of $0.03 \text{ g N} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ there was ammonium oxidation to nitrate (Fig. 4). Only a vestigial quantity of nitrite was observed. Contrary to the longer series, in series II the increase of activated sludge

loading to $0.07 \text{ g N} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ caused (besides nitrate) nitrite accumulation. Nitrite made up about 60% of total N-oxidized forms in the effluent.

At a similar C/N ratio the nitrification efficiency for the longer series was 78.8%. For shorter retention time nitrification efficiency decreased to 55.5%.

Nitrification rate that was estimated on the basis of N-oxidized form changes was different with relation to the rate estimated on the basis of the increase of the sum of nitrite and nitrate in the reactor (Tab. 4).

The difference between accessible amount of nitrogen oxidized forms and real nitrite and nitrate concentration in the reactor during reaction time indicate that it was N-oxides reduction by activated sludge (Fig. 5). Estimated denitrification rate in series I was $0.51 \text{ mg NO}_{x(\text{red})} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ and in series II - $1.56 \text{ mg NO}_{x(\text{red})} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$.

In both series denitrification efficiency remained on the level of 52%. For the longer series activated sludge reduced about $44.7 \text{ mg NO}_x \cdot \text{dm}^{-3}$ of nitrogen oxides. In contrast, for the shorter series only $30.96 \text{ mg NO}_x \cdot \text{dm}^{-3}$ was reduced. The same denitrification efficiency results

Table 4. Nitrification and denitrification rates.

Determination	Unit	Series I	Series II
Nitrification rate	mg NO _x · g ⁻¹ · h ⁻¹	1.12	2.01
Increase of nitrite and nitrate rate	mg NO ₂ ,NO ₃ · g ⁻¹ · h ⁻¹	0.81	1.37
Denitrification rate	mg NO _{x(red)} · g ⁻¹ · h ⁻¹	0.51	1.56

from the fact that nitrogen oxides amount that could be reduced, for series II was less in proportion to this amount in series I.

Efficiency of nitrogen removal by activated sludge during both reduction and biomass synthesis for series I was 52%. At 12-hour retention time despite the amount of nitrogen used for synthesis, increased nitrogen removal efficiency decreased to 43%.

Discussion

Technological research in a single-stage batch reactor with activated sludge showed that under constant oxygen supply to the reactor mixed microorganisms were able to nitrify, denitrify and consume organic matter. Nitrogen removal efficiency from wastewater was 52% for series I and 43% for series II in (depending on retention time). Higher nitrification efficiency was observed when organic carbon loading of activated sludge was 0.11 g COD · g⁻¹ · d⁻¹, ammonium was accessible and the sludge age was 32 days.

Experiment results showed that nitrification efficiency under mentioned conditions was 78.8% and nitrate made up almost 100% of N-oxides. According to Itokawa et al. [11] nitrate accumulation, lack of ammonium in the effluent and the high rate of nitrification were observed when C/N ratio in waste was 2.4 and 3.5. These observations are not confirmed for the shorter series.

When organic carbon loading of activated sludge increased to 0.25 g COD · g⁻¹ · d⁻¹, C/N ratio remained the same 3.5 and at the sludge age of 16 days nitrogen removal efficiency was slowed down. Nitrogen removal decreased because nitrification efficiency decreased. Just only 55.5 % of ammonium was oxidised. The results of Patureau, et al. [2] showed that the inhibition of nitrifying microorganism activity could be caused by higher organic compound levels in the environment.

Nitrite accumulation in the effluent in 12-hour series was observed. According to Münch, et al. [12] the first step of nitrification was not inhibited even at high COD concentration. A high dosage of COD caused nitrite oxidation to be slower, which resulted in nitrite accumulation. The authors indicate that there are groups of microorganisms that are able to carry out shorter nitrification simultaneously with denitrification. Bacteria can oxidise ammonium to nitrite and in the next step they reduce nitrite to nitrous oxide or gaseous nitrogen. This complex process is connected with the inhibition of the second step

of nitrification, which can cause nitrite accumulation.

In both series estimated amount of N-oxidized forms was higher than the sum of nitrite and nitrate concentration in reactor during reaction time. For the longer series nitrification rate in activated sludge 1.12 mg NO_x · g⁻¹ · h⁻¹ was higher than the increase of nitrite and nitrate rate in the reactor - 0.81 mg NO₂,NO₃ · g⁻¹ · h⁻¹. It is suggested that N-oxidized forms were simultaneously reduced by activated sludge. During the initial phase of the reaction (t=0 h to t=4 h) when the oxygen was exhausted, despite the fact that oxygen was constantly supplied to the reactor, total reduction of oxidised nitrogen compounds was observed. From the present investigation it results that although oxygen concentration increase and C/N ratio decline. During the next hours of the experiment denitrification was not inhibited. Denitrification was slower and N-oxidized forms appeared in the reactor.

The research of Huang, et al. [1] indicated that among other parameters (temperature, pH, nitrate concentration) organic carbon concentration (especially C/N ratio) is suggested to be the major factor affecting aerobic denitrification by *Citrobacter diversus*. Several studies have demonstrated that within the optimal range, the higher carbon concentration as energy source the faster denitrification reaction of the aerobic denitrifiers [13]. The results obtained by Huang, et al. [1] showed that the initial denitrification rate increased quickly with increasing C/N ratio from 2 to 4. This is reasonable because the higher the carbon source, the faster the cell growth. Here the maximum denitrification rate was about 5. Further increase in the C/N ratio results in a denitrification rate by *Citrobacter diversus*.

Present investigations showed that C/N ratio at the beginning of the reaction remained on the same level (3.5) although retention time has changed from 24 to 12 hours. After 3 hours of experiment leading C/N ratio in wastewater in the reactor decreased to about 1÷2 during the next hours of the reaction. Denitrification inhibition was not observed, it was only slower than at the beginning.

The accessibility of volatile fatty acids (VFA) is a very important factor during nitrogen removal by activated sludge. Readily biodegradable fractions are known to stimulate nitrogen reduction [14]. This kind of fraction is directly adsorbed by microorganisms and metabolised. It is supposed that the particulate fraction demands initial hydrolysis by extracellular enzymes to volatile fatty acids [10].

Low soluble carbon compound concentration in used wastewater did not cause carbon compound deficits during nitrogen transformation processes in activated sludge. Raw rural sewage contained high levels of particular fraction, expressed as suspended solids. In both series bioassimilable compounds ($C_{pb,COD}$) made up about 84% of the whole amount of suspended solids ($C_{p,COD}$). Particulate fraction was hydrolysed and was used as carbon source (electron donors) for the reduction of N-oxidized form by activated sludge.

This article presented at the same C/N ratio 3.5, an increase of organic carbon loading from the value of $0.11 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ to $0.25 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ did not cause the inhibition of nitrification. Limited accessibility of N-oxidized forms brought about the decrease of their reduction.

Conclusion

- The denitrification process is possible in a single-stage constantly aerated reactor with activated sludge. Obtained denitrification efficiency at C/N ratio 3.5 during both experimental periods was about 50%.
- Using a single-stage aerated reactor with activated sludge permits nitrate substrate elimination from raw rural wastewater by denitrification and biomass synthesis. At a shorter retention time the increase of biomass synthesis was observed (series I - $8.13 \text{ mg} \cdot \text{dm}^{-3}$, series II - $11.26 \text{ mg} \cdot \text{dm}^{-3}$).
- An increase of organic carbon loading from the value of $0.11 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ to $0.25 \text{ g COD} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ decreased denitrification efficiency from 52 % to 43 %. This denitrification decrease is probably caused by the nitrification efficiency decrease (fewer N-oxides were accessible).
- Readily bioassimilable compounds such as volatile fatty acids can be used as a carbon source for denitrification.
- Nitrogen removal in the aerated reactor is more dependent on organic carbon loading than on C/N ratio.

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