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EFFECT OF CARBON SOURCE AND NITRATE CONCENTRATION ON DENITRIFICATION OF HIGH-NITRATE WASTEWATER

The aim of the present work was to study the applicability of the activated sludge from a municipal wastewater treatment plant for high strength nitrate denitrification process. A sequenced batch reactor operated at initial nitrate concentration in the range from 750 to 8500 mg NO_3^- -N/dm³. The effectiveness of various carbon sources on denitrification rate and nitrite accumulation was studied. The average specific denitrification rate was in the range from 58 to 62 mg NO_3^- -N/(g VSS·h) for ethanol, glycerol, molasses and whey. The denitrification with methanol was not effective.

1. INTRODUCTION

The treatment of industrial waste with high nitrate content is considered a major problem for waste purification technologies. Nitrogen-containing compounds are contaminants that can cause serious difficulties in the environment. Excess of nitrates in surface water can lead to the eutrophication of rivers and water bodies and can indirectly cause a lack of oxygen and decline in biodiversity. Nitrates present in higher concentrations in drinking water, also pose potential risks to human and animal health (methemoglobinemia). The World Health Organisation established for nitrates in drinking water the limit 10 mg NO_3^- -N/dm³, which corresponds to about 50 mg NO_3^- /dm³.

The concentration of nitrogen compounds in the majority of industrial wastewater is significantly higher than that of the groundwater and surface water. Wastewater containing high strength nitrates (more than 1000 mg NO_3^- -N/dm³) are generated in large volumes in various industries such as fertilizer, pharmaceutical, nuclear, metal finishing, explosives, dairy [1–3]. Extreme nitrate concentrations are also reported for the wastewater from the nuclear industry – up to 50 000 mg NO_3^- -N/dm³, originating from

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nitric acid used for metal cleaning [1]. Water with high concentrations of nitrates occurs also in wrapping meat, milk processing, refinery or production of synthetic fibres [4]. Other sources are wastewater treatment – regeneration solution from the ion exchange columns, production of nitric acid and additives for fuels and other chemical production [5] or pickling of stainless steel [6].

Physical and chemical processes such as separation by ion exchange or reverse osmosis, reduction with chemical reducing agents, and electro dialysis are effective for nitrate removal in low concentration. Disadvantages associated with these methods are mainly the production of secondary wastes, caused by separating nitrates from the waste stream in the term of more concentrated solution, the additional treatment before disposal is, therefore, indispensable [7].

A method which enables not only separation but the conversion of nitrates to harmless products is biological denitrification. Biological denitrification is based on the transformation of oxidized nitrogen compounds (nitrates and nitrites) by bacteria into harmless nitrogen gas. Biological denitrification is the most economical, effective and environmental approach for removing nitrate from surface water and wastewater.

1.1. CARBON SOURCE

The majority of industrial biological denitrification processes uses heterotrophic denitrifying bacteria requiring an organic carbon source. Heterotrophic biochemical removal of nitrates from industrial wastewater, however, is complicated by their improper composition – high nitrate concentrations, presence of other salts and usually lack organic nutrients and trace elements. Therefore the organic compound used as an energy source for denitrification has to be supplied in the form of exogenous easily degradable substrate. Choice of the carbon source affects the rate and course of the denitrification, its efficiency, the formation of sludge and microbial biomass composition. The whole variety of substrates has been used in many studies mostly methanol [8, 9], acetate [1, 10], ethanol [11, 12], and acetic acid [13, 14]. Numerous different industrial waste products were tested, i.e., molasses [15, 16], glycerol as a byproduct of biofuels production [17–19].

1.2. EFFECT OF NITRATE CONCENTRATION ON BIOLOGICAL DENITRIFICATION

The process of biological denitrification in wastewater treatment has been extensively studied, but there is only a limited number of publications on the denitrification of high-nitrate wastewater [1–3, 9, 13, 20]. A frequent problem associated with denitrification of wastewater with high nitrate content is the accumulation of nitrite as an intermediate reaction product. Glass and Silverstein [1] found that this accumulation was associated with subsequent inhibition of denitrification. There is a lack of information about the dependence of nitrite accumulation on the type of carbon source and its amount.

The aim of the present paper was to investigate the applicability of the activated sludge from a municipal wastewater treatment plant for high rate denitrification processes in the field of water solutions of inorganic compounds. The study was aimed at the influence of carbon source type and initial nitrate concentration on the denitrification course and rate. The denitrification of synthetic wastewater was investigated in a batch reactor.

2. MATERIALS AND METHODS

Experimental set-up. Denitrification was performed in a semi-continuous batch arrangement. Experiments were carried out in a mixing reactor consisting of a closed stirred plastic container placed in a water bath kept at constant temperature 30 ± 1 °C. The suspension was stirred using Heidolph overhead stirrer at 70 rpm. pH, temperature and conductivity were measured continuously and recorded in PC.

The selection of a suitable carbon source was carried out at the beginning of testing. Six different carbon sources were used for the denitrification of $1500 \text{ mg NO}_3^- \text{-N/dm}^3$ in the batch with original activated sludge and COD/N ratio 4. After selection of carbon source, the activated sludge was acclimatized to the nitrate-nitrogen concentration of $1500 \text{ mg NO}_3^- \text{-N/dm}^3$ with each of selected carbon sources over a period of 30 days, sufficient for the stabilization of denitrification rate.

The experimental set-up was the same for all tests. At the start of each batch process, the reactor was filled with 1 dm^3 of sludge and 2 dm^3 of synthetic wastewater. All experiments were carried out in anoxic conditions. At the end of the test (when the concentration of nitrate and nitrites was under the discharge limits), the stirring was turned off, the sludge settled and the supernatant was cautiously drawn off and replaced by prepared synthetic wastewater. Biomass concentration in the reactor was kept between 3.5 and 4 g VSS/dm^3 . The initial pH was adjusted to 7.5–8 by addition of diluted H_2SO_4 or NaOH.

The experiment under each set of conditions (COD/N ratio, NO_3^- concentration, the carbon source) was repeated 25 times (tests of initial nitrate concentration effect 3 times), the results presented in tables and figures are the average values of these 25 experiments.

Activated sludge. Microorganism consortia as activated sludge are the most frequently used microorganisms for wastewater treatment. The activated sludge was taken from municipal wastewater treatment plant of the town Ústí nad Labem (Czech Republic). Biomass consisted of strong irregular flocs with frequent occurrence of biomass fragments smaller than $50 \text{ }\mu\text{m}$. Large flocs were agglomerated. Zooglea were mostly

small with compact structure. Higher organisms were represented by flagellates, amoebes, arcellinida and diatoms. Frequently, filamentous organisms were observed, with dominant *Microthrix parvicella*, *Type 0092* and *Nostocoida limicola* (Fig. 4A).

Characteristics of wastewater and carbon sources. For the experiments, the synthetic wastewater was used with the concentration of nitrate from 5 to 50 g $\text{NO}_3^-/\text{dm}^3$, which corresponds to NO_3^- -N concentration in batch from 1125 to 11 850 mg NO_3^- -N/ dm^3 . The composition of synthetic wastewater simulated the composition of neutralized stainless steel pickling rinse water, nitrate was dosed in the form of NaNO_3 , phosphorous in the form of KH_2PO_4 . The microelements: Fe 4 mg/ dm^3 , Mn 3 mg/ dm^3 , Cu 0.8 mg/ dm^3 , Co 1.3 mg/ dm^3 , Zn 1 mg/ dm^3 were added in all cases. The composition of synthetic wastewater with various initial NO_3^- -N concentration is shown in Table 1.

Table 1

The composition of the synthetic wastewater [mg/ dm^3]

Initial NO_3^- -N in batch	NO_3^- -N in synthetic wastewater	P	Mg	Ca
750	1125	112.5	10	45
1250	1875	187.5		
1500	2250	225		
1800	2700	270		
2500	3750	375		
2800	4200	420		
3900	5850	585		
4500	6750	675		
5300	7950	795		
7900	11850	1185		

The carbon sources used were ethanol (96%), glycerol (99.5%), methanol (99.5%), sucrose (beet sugar), and two alternative carbon sources, molasses (the mixture of molasses and sugar syrup) and acid whey. The initial COD/N ratio in the batch was set at the selected value in the range from 3.5 to 8 by adding one of the above-mentioned carbon sources. The characteristics of the carbon sources used are given in Table 2. Sucrose is solid and molasses was very viscous in nature, hence the COD was determined in g O_2/g sample and this carbon sources were dosed by weighing.

Analytical methods. Liquid samples were centrifuged 5 min at 9000 rpm and obtained supernatant was used for nitrate and nitrite analysis. Nitrate concentration was determined by using nitrate ion-selective electrode (Elektrochemické detektory, s.r.o). Nitrite concentration was measured spectrophotometrically by the sulfosalicylate method

at 540 nm on spectrophotometer UNICAM 86 25 in 1 cm quartz cuvette. Volatile suspended solid (VSS) was monitored gravimetrically according to the standard methods EN 12879:2000. The chemical oxygen demand (COD) was measured by the chromate method according to ISO6060:2008.

Table 2

The composition and dosage of carbon sources at the initial concentration 1 500 mg and COD/N of 4

Carbon source	COD		P [mg/kg]	Ca [g/kg]	Mg [mg/kg]	Dosage of carbon source model wastewater	
	[g O ₂ /dm ³]	[g O ₂ /kg]				[cm ³ /dm ³]	[g/dm ³]
Ethanol (96%)	1615		–	–	–	3.7	
Glycerol (99.5%)	1524		–	–	–	3.9	
Sucrose		1116	–	–	–		5.3
Methanol (99.5%)	1179		–	–	–	5.1	
Molasses		682	24	1.19	106		8.8
Whey	66.2		665	2.67	9	90.6	

Kinetics. Nitrate and nitrite reduction follow in biological denitrification zero-order kinetics [1, 3, 9]. The relation of nitrate-nitrogen (NO₃⁻-N) concentration and time of reaction provided (in a majority range of curves) a linear fit. Thus the volumetric denitrification rate of nitrate-nitrogen reduction was assessed by the linear regression coefficient of the NO₃⁻-N concentration decrease [3, 6]. Specific denitrification rates were calculated by dividing the volumetric denitrification rates by the VSS concentrations determined at the beginning of each test.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF CARBON SOURCE ON BIOLOGICAL DENITRIFICATION

Six different carbon sources were used to acclimatize the biomass. Based on the literature data, a COD/N ratio of 4 was used. Figure 1 shows time courses of the starting batch tests of the denitrification process with original activated sludge before acclimatization with different carbon sources. The initial nitrate-nitrogen concentration in the batch was in all cases ca. 1500 mg NO₃⁻-N/dm³ and VSS concentrations were in the range from 3.5 to 4 g/dm³.

Complete denitrification was observed for all carbon sources used except for methanol and sucrose. The time needed for complete removal of nitrate and nitrite reached 43.2 h with whey, 52.4 h with ethanol, 69.2 h with glycerol and 90.3 h with molasses. The effluent

concentration of nitrate-nitrogen was below 20 mg NO_3^- -N/dm³ and that of nitrite-nitrogen below 2 mg NO_2^- -N/dm³ mg in the case of ethanol, molasses, glycerol, and whey.

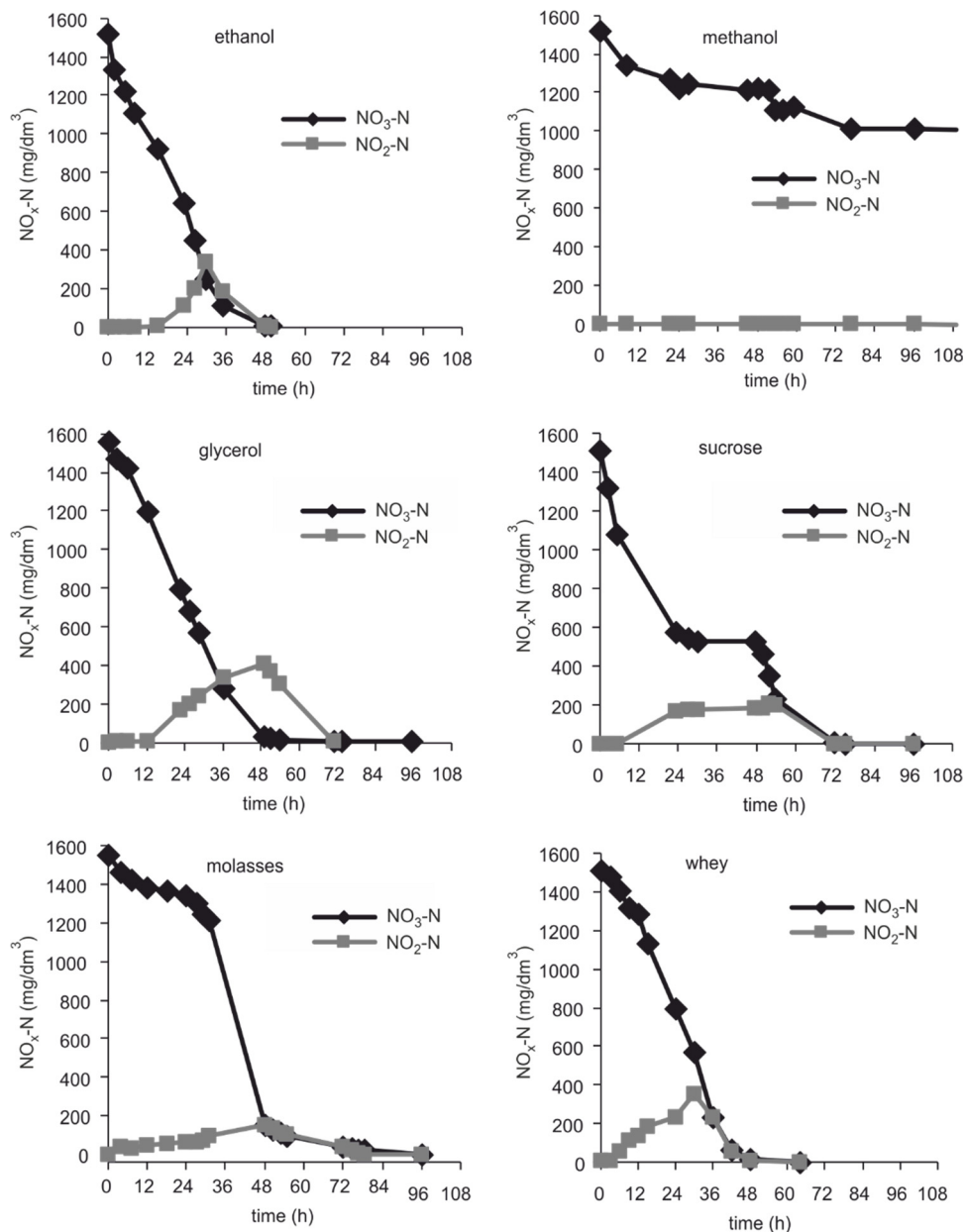


Fig. 1. Nitrate-nitrogen and nitrite-nitrogen profiles during denitrification of 1500 mg NO_3^- -N/dm³ in the start-up test with various carbon sources, COD/N 4, 30 °C

In the test with sucrose, the process of denitrification stopped after 24 h, the concentration of NO_3^- -N and NO_2^- -N remained constant. After the addition of extra sucrose (48 h after the process started), the denitrification continued, and nitrate and nitrite were completely removed.

In the case of using methanol as a carbon source, the long adaptation period was required. The denitrification using methanol without previous adaptation of bacteria to this carbon source, using the same procedure as for other sources, was not effective, the reduction of nitrate was very slow and even after 100 h nitrate was not removed. The formation of nitrite was insignificant, the maximum nitrite-nitrogen concentration was 0.19 mg NO_2^- -N/dm³. COD measurement (Table 3) showed that methanol was consumed only by 28.5%. This observation is similar to that of Akunna et al. [17] but methanol was successfully used by many other, above mentioned researchers. Very low denitrification rate can be in our case caused by the lack of bacteria capable exploit methanol, and by insufficient time for their multiplication. The specific growth rate of methylotrophic bacteria is lower than those using other carbon sources, resulting in insufficient denitrification kinetics and longer adaptation period [21].

Table 3

The course of the denitrification in the start-up batch with various carbon sources

Carbon source	$r_{\text{NO}_3^- \text{-N}}$ [mg/(g VSS·h)]	Effluent COD [mg O ₂ /dm ³]	Time for NO ₃ ⁻ N removal [h]	Time for N _{tot} removal [h]
Ethanol	9.3	447	40.8	52.4
Methanol	1.6	4290	–	–
Sucrose	7.9	143	–	–
Glycerol	7.2	478	45.6	69.2
Molasses	5.5	751	69.8	90.3
Whey	8.1	251	46.7	43.2

Initial nitrate-nitrogen content 1500 mg, initial pH 7.5, temperature 30 °C, COD/N 4.

Transient accumulation of nitrite was observed in all tests with original activated sludge (before acclimatization) at COD/N 4, except test with methanol. The formation of nitrite was accompanied by a decrease in pH from the original 7.5 to 6.5–7. Subsequent decomposition of nitrite led to an increase of pH to 8.5. Maximum transient nitrite concentrations reached in the case of glycerol 400 mg NO_2^- -N/dm³, with ethanol 336 mg NO_2^- -N/dm³, with molasses 152 mg NO_2^- -N/dm³, with whey 351 mg NO_2^- -N/dm³ and with sucrose 200 mg NO_2^- -N/dm³. The specific denitrification rates in starting batches with different carbon sources increased in the order methanol < molasses < glycerol < sucrose < whey < ethanol (Table 3). The concentration of COD in the effluent was the highest in the case of molasses 751 mg O_2 /dm³. The real consumption of carbon source calculated from

COD concentration in effluent correspond to COD/N ratio of 3.7 for ethanol and glycerol, 4.7 for sucrose, 3.8 for whey and 3.5 in the case of molasses.

The further experiments were carried out with ethanol, glycerol, molasses and whey. The activated sludge was acclimatized to nitrate-nitrogen concentration 1500 mg NO_3^- -N/dm³ with each of these carbon sources for 30 days. The acclimation and growth of denitrifying microorganisms resulted in a significant decrease of the time needed for complete denitrification during subsequent batches for all tested carbon sources (ethanol, glycerol, molasses and whey) (Table 4). The time necessary for complete removal of total nitrogen (nitrate- and nitrite-, time N_{tot}) was 8.3 h for ethanol and whey, 7.1 h for molasses and 10.4 h for glycerol. In all the tests, however, the formation of nitrites as intermediate of nitrate removal was observed. The maximum nitrite-nitrogen concentration was observed after ca. 5 h for ethanol, molasses and whey and reached 544 mg NO_2^- -N/dm³ in the case of ethanol and ca. 400 mg NO_2^- -N/dm³ with whey and molasses. The nitrite-nitrogen accumulation reached the lowest value under glycerol as a carbon source, only 200 mg NO_2^- -N/dm³ after 6 h. The average values of specific denitrification rate presented in Table 4 are very similar for all carbon sources used.

Table 4

The average values of denitrification rate in batches with acclimatized sludge for various carbon sources

Carbon source	$r_{\text{NO}_3^- \text{-N}}$ [mg/(g VSS·h)]	NO_2^- -N accumulation [mg/dm ³]	Final effluent COD [mg/dm ³]	Time for N_{tot} removal [h]
Ethanol	62.0	544	449	8.3
Glycerol	58.2	200	393	10.4
Molasses	60.5	368	909	7.1
Whey	62.3	412	665	8.3

Initial nitrate-nitrogen content 1500 mg, initial pH 7.5, temperature 30 °C, COD/N 4.

The real consumption of COD after acclimatization of activated sludge was about 3.7 g COD/g N for ethanol and glycerol, alternative carbon sources were consumed only in ratio 3.4 g COD/g N for molasses and 3.6 g COD/g N for whey.

In terms of rate and efficiency of nitrate removal, whey and molasses appear to be suitable substrates for biological denitrification. Whey contains a number of compounds with positive effect on microorganisms: lactose (ca. 70–80% of dry matter), proteins and vitamins (group B, E, C, A, biotin), minerals (phosphates, chloride, calcium, sodium, potassium, magnesium), along with traces of fat. The disadvantage of whey and molasses as alternative carbon sources is the introduction of other substances to the system. In experiments with molasses, a slightly yellowish colour of effluent was observed. Molasses contains except for invert sugars (saccharose, glucose, and fructose) and other

components, also a variety of coloured organic compounds such as melanoidine, caramel and polyphenolic compounds. The coloured organic compounds are probably not readily decomposed by the microorganisms and thus remain in the effluent similarly as proteins originated from whey. Further treating of effluent is therefore in the case of these alternative carbon sources necessary.

3.2. EFFECT OF COD/N RATIO ON BIOLOGICAL DENITRIFICATION

Effect of COD/N ratio was tested with ethanol as the carbon source. The courses of nitrogen compounds (nitrate and nitrite) transformation during denitrification with various COD/N ratio of ethanol as a carbon source are shown in Fig. 2.

The COD/N ratio 3.5 was insufficient for complete nitrogen (nitrate and nitrite) removal (Fig. 2a). The concentration of nitrite-nitrogen in the effluent was 115 mg NO_2^- -N/dm³, the concentration of nitrate-nitrogen 16 mg NO_3^- -N/dm³. The accumulation of nitrite-nitrogen was observed under COD/N ratios from 3.5 to 6 (Figs. 2a–e). The rate of nitrite-nitrogen formation decreased with increasing COD/N and no accumulation of NO_2^- -N was observed under COD/N of 8 (Fig. 2f).

These results are in the contrary to that of Ge et al. [22], who reported positive relationship between nitrite accumulation rate and COD/N ratio in the range of 1–25 with acetate, methanol and glucose as carbon sources. Table 5 summarizes the denitrification rates with various COD/N.

Table 5

Effects of COD/N ratio on the denitrification rate

COD/N	$r_{\text{NO}_3^-}$ [mg/(g VSS·h)]	NO_2^- -N accumulation [mg/dm ³]	Final effluent COD [mg/dm ³]	Time for NO_3^- N removal [h]	Time for N_{tot} removal [h]
3.5	70	789	21	5.8	–
4	62	544	449	6.1	8.3
4.5	72	615	538	5.3	7.6
5	74	551	617	5.2	7.4
6	65	113	2254	4.5	5.6
8	50.5	5	5510	7.3	7.3

The source of COD ethanol, temperature 30 °C, initial pH 8.0, initial NO_3^- -N concentration 1500 mg/dm³.

The actual consumption of COD calculated from residual COD in the effluent reached 3.7 in the case of initial COD/N of 4, which is in good agreement with Glass and Silverstein [1] and Foglar et al. [20]. With increasing availability of carbon source (COD/N of 5 – 8), the real consumption increased to COD/N of about 4.5. However,

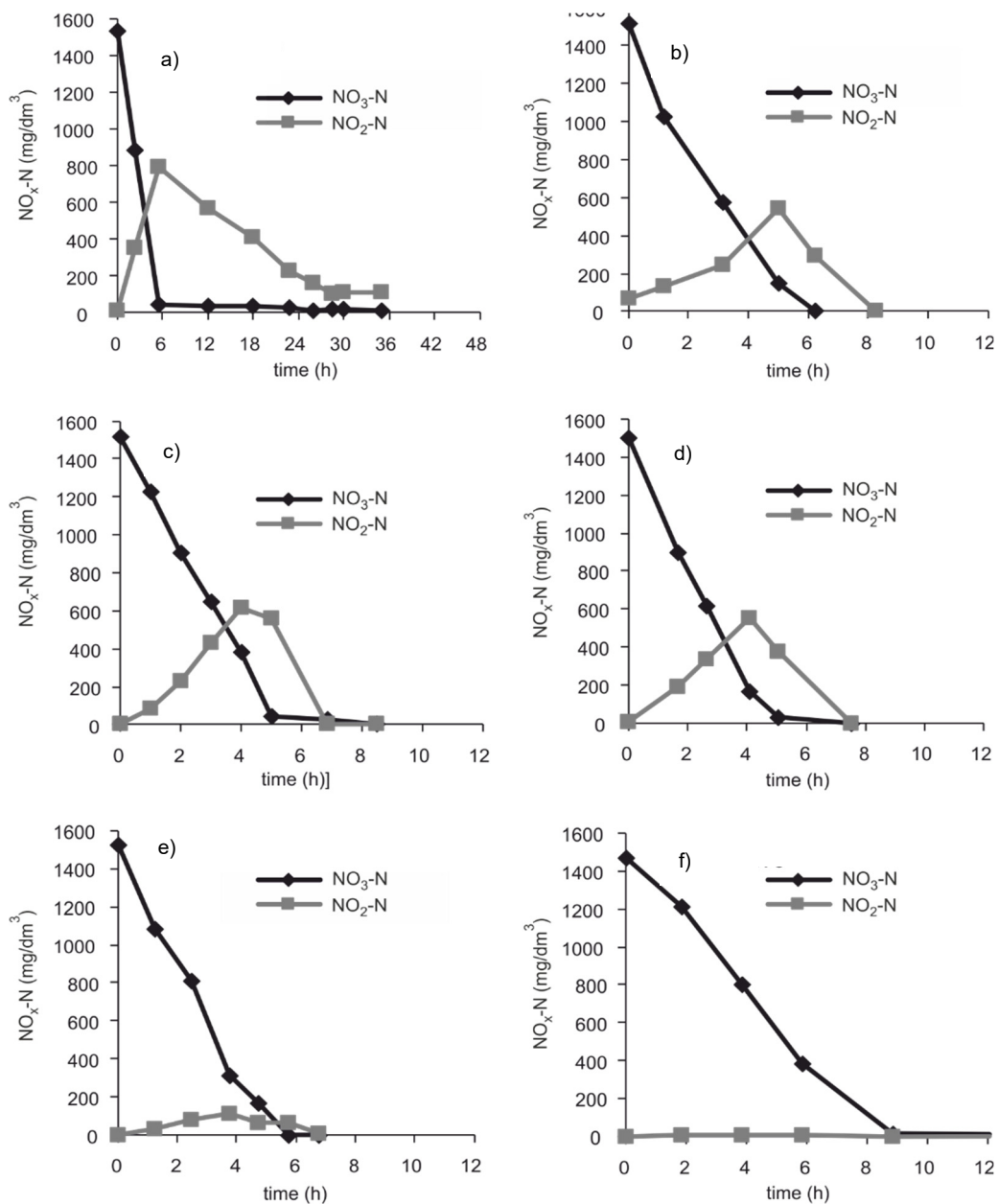


Fig. 2. Nitrate-nitrogen and nitrite-nitrogen profile during denitrification of $1500 \text{ mg NO}_3\text{-N/dm}^3$ at different COD/N ratios; a) COD:N 3.5, b) COD:N 4.0, c) COD:N 4.5, d) COD:N 5.0, e) COD:N 6.0, f) COD:N 8.0

the concentration of residual COD in final effluent is in these cases high above the discharge limits, the further processing of the output stream would therefore be necessary.

3.3. EFFECT OF NITRATE CONCENTRATION ON BIOLOGICAL DENITRIFICATION

The nitrate concentrations in industrial wastewater vary widely even in one type of wastewater over time. The effect of input nitrate content was studied by increasing the nitrate-nitrogen concentration in synthetic wastewater stepwise from 5 to 50 g NO_3^- -N/dm³, which corresponds to 750 and 7900 mg NO_3^- -N/dm³ in batch. The tests were performed with carbon sources: whey, molasses and ethanol.

Figures 3a–e show that the acclimatized activated sludge was capable of removing of up to 2800 mg NO_3^- -N/dm³ from synthetic wastewater during 24 h under the COD/N ratio of 4 and carbon source ethanol. The time required for denitrification of initial nitrate-nitrogen concentration of 3 900 mg NO_3^- -N/dm³ and 4 500 mg NO_3^- -N/dm³ was 30.2 and 100 hours, respectively. The maximum nitrite-nitrogen concentration picked about 20% of initial nitrate-nitrogen concentration when initial nitrate-nitrogen concentration was in the range from 1500 to 3800 mg NO_3^- -N/dm³ but increased to 70% of initial NO_3^- -N concentration when it was 4500 mg NO_3^- -N/dm³ and to nearly 100% at the initial nitrate-nitrogen concentration of 5300 mg NO_3^- -N/dm³. Mohan et al. [10] reported that the accumulation of nitrite can be caused, apart from high dissolved oxygen level, carbon source limitation and absence of true denitrifiers, by reduced activity of nitrite reductase in the presence of nitrate. Nitrate is preferred as an electron acceptor over nitrite when both are presented.

With the initial nitrate-nitrogen concentration of ca. 5300 mg NO_3^- -N/dm³, the nitrate-nitrogen concentration was lowered to 150 mg NO_3^- -N/dm³ after 118 h, but the concentration of total nitrogen (nitrate- and nitrite-nitrogen) remained nearly constant (Fig. 3f). With decreasing nitrate-nitrogen concentration, the nitrite-nitrogen concentration increased linearly to the maximum of 5193 mg NO_2^- -N/dm³, which seems to inhibit completely the second step of denitrification – the reduction of nitrite.

Extreme concentrations of nitrate/nitrite together with nearly anaerobic conditions, caused by a high load of sludge, influenced the morphology of activated sludge. While the sludge flocs kept the relatively large size and usually contained compact core, the external structure of flocs was thin. This was an apparent cause of the marked occurrence of free bacteria in the supernatant (decantate) and the deterioration of the outflow in parameters such as turbidity (an increase from 30 NTU to more than 140 NTU) or COD (an increase from 450 to 820 mg O_2 /dm³). An undesirable high content of zoogeal colony was observed in sludge from these batches (Fig. 4b). The excessive occurrence of these extracellular polymers was observed at anoxic or toxic conditions and led to poor sludge settling.

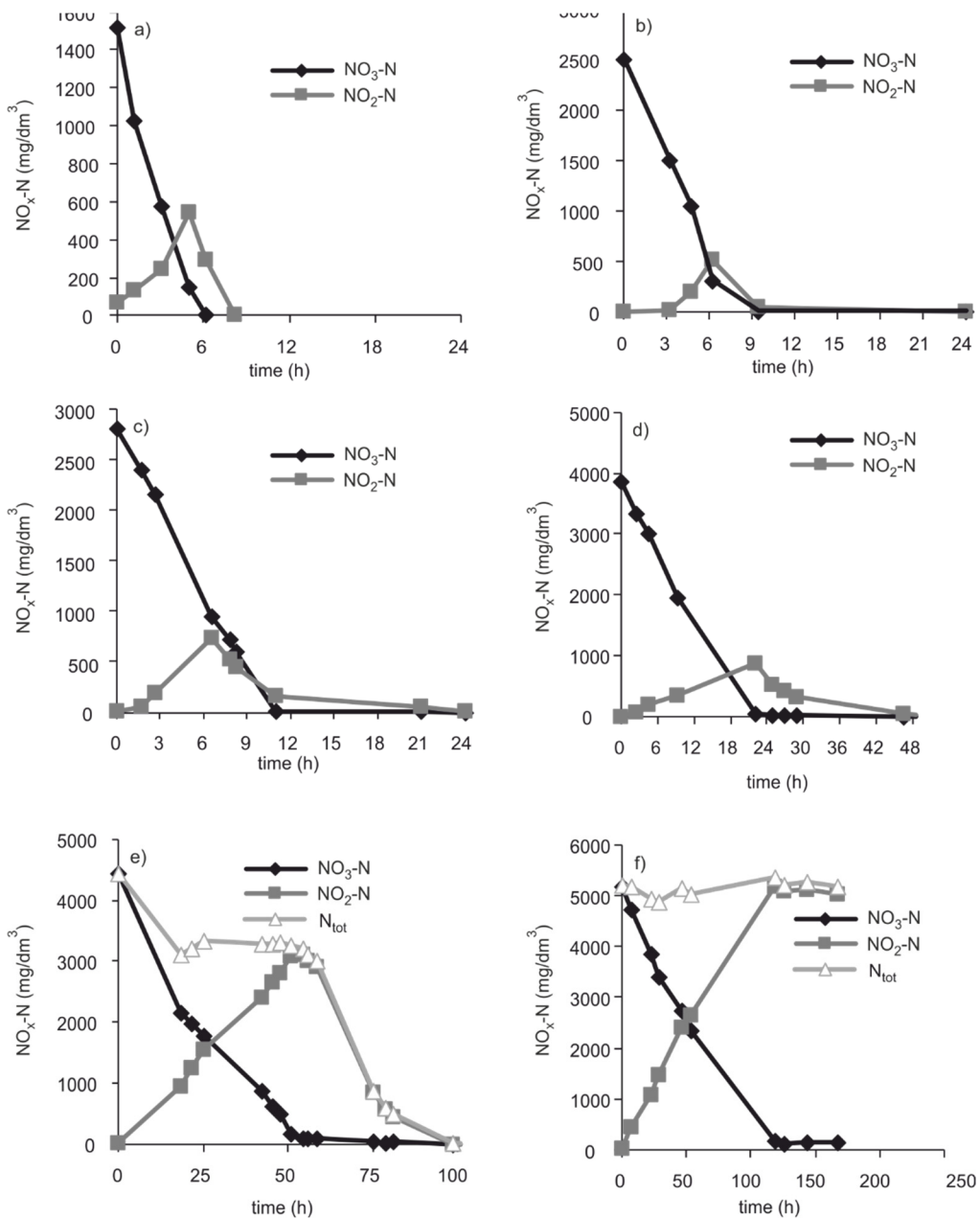


Fig. 3. Nitrate-nitrogen and nitrite-nitrogen profiles during denitrification of various influent concentration of NO_3^- -N/dm³, COD/N 4, carbon source ethanol

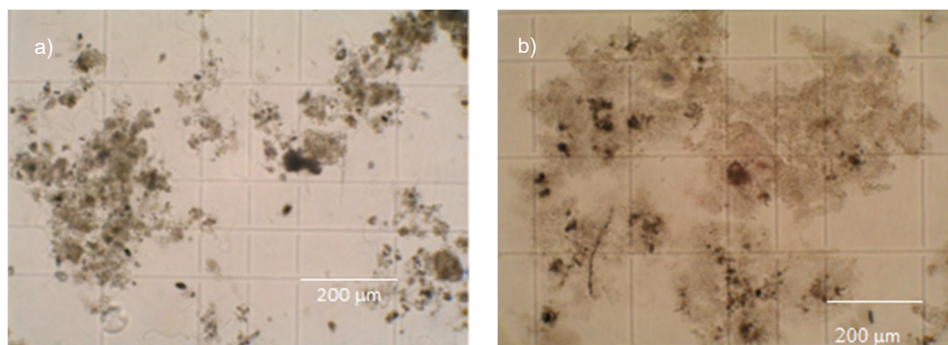


Fig. 4. Original activated sludge (a) and activated sludge after 2 weeks of denitrification (b)

Table 6

Effect of initial nitrate-nitrogen concentration and carbon source type on denitrification kinetics and nitrite accumulation at 30 °C, COD/N 4, initial pH about 8

Carbon source	Initial concentration [mg/dm ³]	NO ₂ ⁻ -N accumulation		$r_{\text{NO}_3^- \text{-N}}$ [mg/(g VSS·h)]	Time for N _{tot} removal [h]
		Amount [mg/dm ³]	Time [h]		
Molasses	1250	360	2.3	60.5	5.9
	1800	416	5.5	74.9	7.1
	2500	996	6.0	100.2	7.2
	2800	933	6.3	92.0	10.5
Whey	1250	413	5.7	63.7	7.4
	1800	449	6.5	62.2	8.3
	2500	398	6.8	92.1	9.0
	2800	432	6.7	89.5	9.0
Ethanol	750	339	2.1	58.1	6.9
	1500	447	5.0	62.0	8.3
	2500	512	6.2	90.3	8.3
	2800	558	21.0	74.2	10.1
	3900	887	24.8	42.3	30.2
	4500	3142	54.2	18.7	100
	5300	5193	118.2	–	–

The kinetic parameters of denitrification with ethanol, molasses and whey, presented in Table 6, indicated the similar trend of increasing the denitrification rate with increasing initial nitrate concentration up to 2800 mg NO₃⁻-N/dm³. The initial nitrate-nitrogen concentration above 3900 mg NO₃⁻-N/dm³, tested only under ethanol as a carbon source, resulted in a decrease in denitrification rate. The effluent concentration of nitrite-nitrogen was in all cases (except the initial concentration 5300 mg NO₃⁻-N/dm³) below 5 mg NO₂⁻-N/dm³ and that of nitrate-nitrogen below 20 mg NO₃⁻-N/dm³.

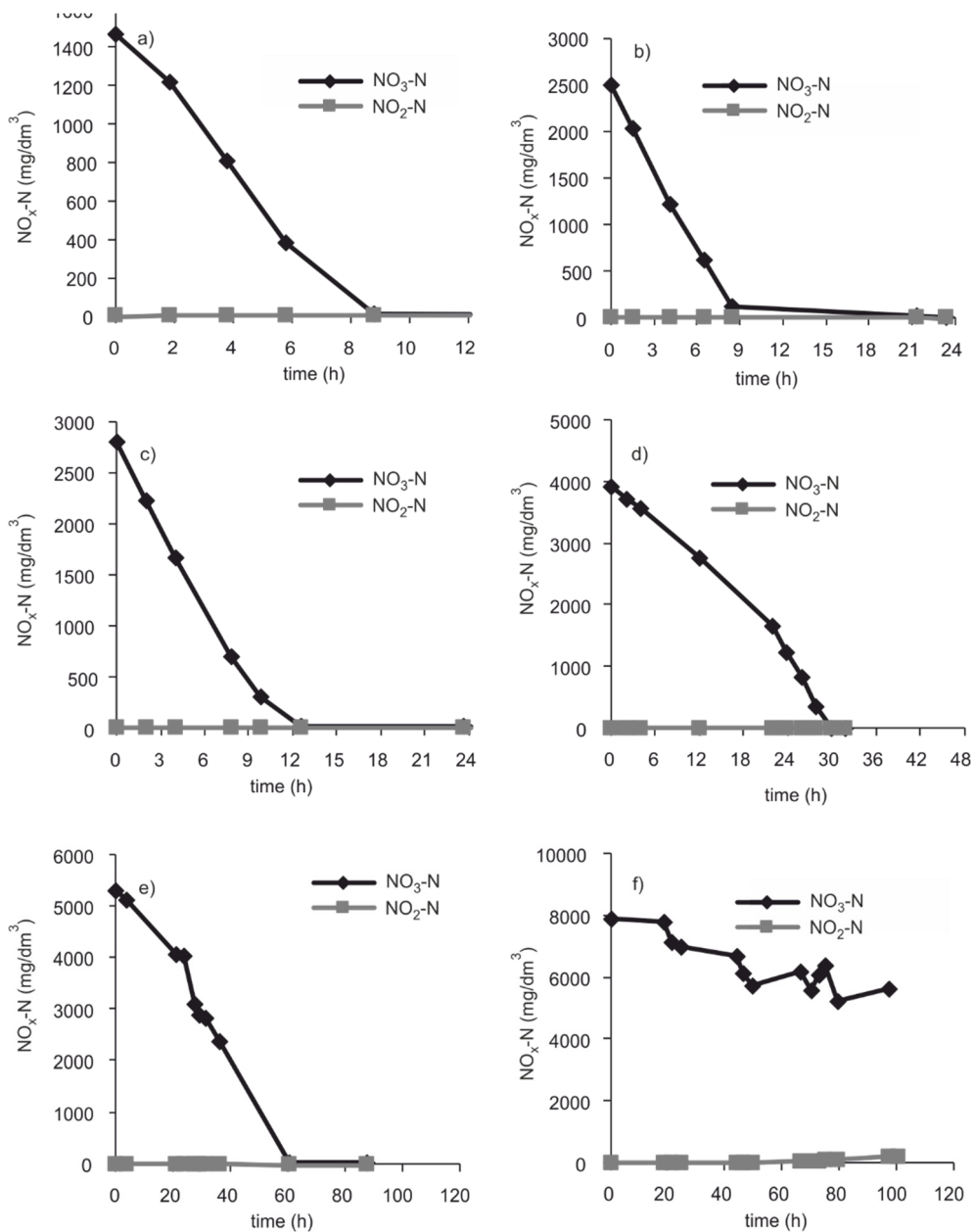


Fig. 5. Nitrate-nitrogen and nitrite-nitrogen profiles during denitrification at various influent concentrations $\text{NO}_3^- \text{-N/dm}^3$, COD/N 8, carbon source ethanol

The course of denitrification under COD/N of 8 was characterized by almost no nitrite-nitrogen accumulation up to initial nitrate-nitrogen concentration 5300 mg

NO_3^- -N/dm³ (Fig. 5), in most cases, the nitrite-nitrogen concentration was under 2 mg NO_2^- -N/dm³ during the whole tests. As shown in Fig. 5, complete inhibition of denitrification took place at the initial nitrate-nitrogen concentration of 7900 mg NO_3^- -N/dm³. These results indicate that nitrite production was insignificant, and that the denitrification was not inhibited by nitrite ions in this case. It is more likely that the activity of microorganisms was suppressed by the high ionic strength of the solution.

Table 7

Effect of initial nitrate-nitrogen concentration on the denitrification kinetics and on nitrite accumulation

Initial NO_3^- -N concentration [mg/dm ³]	Maximum NO_2^- -N concentration [mg/dm ³]	$r_{\text{NO}_3^-}$ [mg/(g VSS·h)]	Time for N_{tot} removal [h]
750	2.37	58.1	7.6
1500	0.41	49.2	7.8
2500	0	88.0	8.1
2800	0	75.3	9.8
3900	6.77	22.1	32.4
5300	1.4	17.0	64.9
7900	215 ^a	4.9	–

^aThe NO_2^- -N concentration was still increasing.

COD/N 8, 30 °C, initial pH about 8.

The results obtained at COD/N ratio of 8 with ethanol as carbon source (summarized in Table 7) are completely different from those observed by Mohan et al. [10] with carbon source acetate or Ge et al. [22] with acetate, methanol and glucose. These authors reported increasing nitrite-nitrogen accumulation with increasing COD/N ratio at input nitrate-nitrogen concentration increasing from 677 mg to 2710 mg [10] and 40 mg NO_3^- -N/dm³ [22], respectively. On the contrary Elefsiniotis and Li [23] observed no nitrite accumulation at C/N ratio 2 and 4 with acetic acid at initial pH of 6.5, but in this case the input nitrate-nitrogen concentration was only 200 mg NO_3^- -N/dm³.

The elevated COD/N ratio of 8 was beneficial for disappearance of nitrite accumulation, but COD concentration in effluent exceeded 10 g O_2 /dm³, the real consumption was between 4.5 to 5 g COD/g. The time for complete denitrification at COD/N ratios of 4 and 8 was almost the same for initial nitrate concentration up to 3900 mg/dm³ NO_3^- -N.

The difference between our and published results of denitrification with ethanol and with acetate [10] can be caused by the differences in metabolisms of the both carbon sources. Denitrification rate with acetic acid/acetate is referred to be higher than that of

ethanol, because acetate can be directly used in carboxyl cycle, while ethanol has to be converted to acetate at first [11].

4. CONCLUSIONS

Batch activated sludge denitrification process was used for denitrification of synthetic wastewater with nitrate content above 5 g/dm^3 ($750 \text{ mg NO}_3^- \text{-N/dm}^3$ in batch) simulating neutralized rinse water from stainless steel pickling. After acclimatization period was the activated sludge capable to completely remove the initial concentration up to $3\,900 \text{ mg NO}_3^- \text{-N/dm}^3$ at COD/N ratio of 4. The denitrification rates were almost the same for all the carbon sources under study at initial nitrate-nitrogen concentration $1500 \text{ mg NO}_3^- \text{-N/dm}^3$ about $62 \text{ mg NO}_3^- \text{-N/(g VSS}\cdot\text{h)}$ under ethanol and whey, $60.5 \text{ mg NO}_3^- \text{-N/(g VSS}\cdot\text{h)}$ under molasses and $58.2 \text{ mg NO}_3^- \text{-N/(g VSS}\cdot\text{h)}$ under glycerol. The denitrification with methanol as carbon source was not effective.

Nitrite accumulation was observed for all carbon sources tested at COD/N ratio of 4. The tests with ethanol at COD/N ratio of 8 showed no nitrite accumulation up to initial nitrate-nitrogen concentration $5300 \text{ mg NO}_3^- \text{-N/dm}^3$.

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