Nitrogen Removal from Municipal Landfill Leachate

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The aim of this work was to investigate the efficiency of nitrogen removal from municipal landfill leachate with a microbial culture in 3 L batch bioreactor. The microbial culture, originating from landfill leachate, was prepared by an enrichment culture technique. Organic compounds measured as chemical oxygen demand (COD) of 400–600 mg L^{-1} and high concentrations of ammonium nitrogen (NH_4^+-N) 270–312 mg L^{-1} characterized the landfill leachate as a mature leachate. The rate of ammonium nitrogen removal was 24.5 mg NH_4^+-N L^{-1} h^{-1}. Nitrification rate was 20.1 mg NO_x^--N L^{-1} h^{-1}. Denitrification was performed with the addition of sodium acetate as external carbon source in ratio C/N 2 and 4. Sodium acetate was insufficient for complete denitrification at C/N 2. Complete denitrification at C/N 4 was performed at denitrification rate 8.3 mg NO_3^--N L^{-1} h^{-1}.

Key words:
Denitrification, landfill leachate, microbial culture, nitrification

Introduction

The landfill leachate is a highly variable and heterogenous mixture of high strength organic and inorganic contaminants including, among others, humic acids, xenobiotic organic compounds (XOCs), ammonia nitrogen, heavy metals and other inorganic salts.[1] Due to the toxicity and potential risk to surrounding soil, ground or surface waters, landfill leachates become a major pollution hazard and must be collected and appropriately treated before being discharged into the environment.[2–8] Many factors affect the quality of leachate, such as age, precipitation, seasonal weather variation, waste type and composition.[1,9] The chemical composition of leachate varies greatly depending on the age and maturity of the landfill site. An immature leachate contains high strength organic compounds and ammonia nitrogen, while a mature leachate contains relatively low concentrations of degradable organic matter but high concentrations of ammonia nitrogen.[1,9] The ammonia nitrogen constitutes a major long-term pollutant in landfill leachate.[1] Biological nitrogen removal through the process of nitrification and denitrification is usually suggested for treatment of landfill leachate with low COD/N ratio.[10–13] Ammonia nitrogen present in mature landfill leachate in high concentrations causes difficulties in their biological treatment because of the inhibition of ammonia oxidizing and nitrite oxidizing bacteria by free ammonia (FA) and nitrous acid (FNA).[14,15] Also, the low COD/N ratio, which is a characteristic of mature leachate, reduces the efficiency of denitrification due to the limited level of biodegradable organics and a supplementary source of organic carbon is required to ensure adequate denitrification. Different external carbon sources (acetate, methanol, ethanol, glucose, maltose and a brewery yeast waste) have been tested for denitrification.[10–13,16–20]

The aim of this study was to investigate the efficiency of biological nitrogen removal from mature municipal landfill leachate. Also, the purpose of this study was to determine the ability and activity of microbial culture isolated from the environment – leachate for the processes of nitrification and denitrification. Because of no biodegradable organic compounds in the leachate, sodium acetate as an external carbon source was used for denitrification.

Materials and methods

Landfill leachate

The leachate used in this study had the typical characteristics of a mature landfill. It was collected from a municipal landfill, which was in operation

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from 1998 to 2008. The total average amount of solid waste deposited in the landfill per year was 37325 m^3. Periodically, the hazardous wastes were dumped in the leachate area. The leachate was collected monthly and stored in 10 L polyethylene containers at 4 °C before use. The composition of the leachate used in the experiments is shown in Table 1. The BOD/COD ratio < 0.2 indicated that the leachate was low in biodegradability. For that reason, sodium acetate was added as a carbon source for denitrification.

T a b l e 1 – *Physical/chemical analysis of landfill leachate*

Parameter	Value
pH	8–8.5
COD/mg L^{-1}	400–600
BOD/mg L^{-1}	67–108
Total N/mg L^{-1}	282–325
NH$_4^+$-N/mg L^{-1}	270–312
NO$_3^-$-N/mg L^{-1}	1.4–2.7
NO$_2^-$-N/mg L^{-1}	0–0.13
PO$_4$-P/mg L^{-1}	12.8–16
As/µg L^{-1}	454
Cd/µg L^{-1}	2.6
Co/µg L^{-1}	< 200
Cr/µg L^{-1}	559
Cu/µg L^{-1}	3140
Fe/µg L^{-1}	19600
Mn/µg L^{-1}	3300
Total PAH/mg L^{-1}	< 0.0002
BTEX/µg L^{-1}	< 5
PCB/µg L^{-1}	< 2
Organochlorine pesticides/µg L^{-1}	< 10
Phenols/mg L^{-1}	0.016
Mineral oils/mg L^{-1}	< 0.005

Microbial culture

In this study, for landfill leachate nitrification and denitrification, a mixed microbial culture was used. The mixed microbial culture was prepared by an enrichment culture technique from landfill leachate sludge collected from the channel through which the landfill leachate flows.

The enrichment of mixed microbial culture was performed in Erlenmeyer flasks (500 mL, with working volume of 100 mL) on rotary shaker

(Certomat IS, B. Braun, Biotechnology International) at 150 rpm and room temperature, and with landfill leachate as a source of ammonium nitrogen (100 mg NH$_4^+$-N L^{-1}). Every 3–4 days, about 15 % of the biomass was inoculated into the landfill leachate (100 mg NH$_4^+$-N L^{-1}). After two months of cultivation, the biomass as inoculum was prepared and used in all experiments.

Batch bioreactor

Nitrification and denitrification experiments were performed in laboratory-scale batch bioreactor (volume of 3 L, working volume of 2 L). Dissolved oxygen was supplied using porous diffusers, placed at the bottom of reactor. For determining of dissolved oxygen (DO), pH and temperature, the electrodes were placed into the bioreactor. After nitrification, the aeration was stopped, biomass settled, supernatant decanted and landfill leachate was added and nitrification was repeated, or after nitrification sodium acetate was added and denitrification was performed at anoxic conditions. The anoxic conditions were maintained by mixing. For denitrification, different dosages of sodium acetate were added. Sodium acetate dosage per nitrate concentration at the beginning of denitrification was 4.95 and 9.9 mg COD per mg NO$_3^-$-N for C/N ratio 2 and 4, respectively. All experiments were performed with biomass concentration expressed as MLSS approximately 1.6 g L^{-1}. Experiments were performed in triplicate and results were presented as mean values ± standard deviation. Also, control experiments without inoculations were performed.

Analytical methods

Total nitrogen (N-tot), ammonium nitrogen (NH$_4^+$-N), nitrite nitrogen (NO$_2^-$-N), nitrate nitrogen (NO$_3^-$-N) and phosphorous orthophosphate (PO$_4$-P) were analyzed photometrically (Merck Spectroquant® Vega 400) with standard kits (Merck-Spectroquant). COD was determined by closed reflux method (5220 C), BOD by dilution method and biomass concentrations were measured as mixed liquor suspended solids (MLSS) by method 2540D, all in accordance with Standard Methods.[21] The pH value and temperature were measured using an electrode connected to a pH-meter (WTW 320, Germany). DO was determined by oximeter WTW oxi-320 (WTW, Germany). Concentrations of metals, total PAHs, PCB, organochlorine pesticides, phenols and mineral oils were determined according to the Croatian legislation for wastewater.[22] Before all analysis, the samples were filtered through filter pore size 0.45 µm.

All calculations and data analysis were performed in Microsoft Excel 2000 (9.0.3821 SR-1). For all calculated reaction rates during nitrification and denitrification the correlation coefficients were higher than 0.98.

Results and discussion

The efficiency of biological nitrogen removal from landfill leachate depends on various factors such as landfill leachate composition, COD/N ratio, biodegradable organic compounds, microbial culture composition and process parameters (dissolved oxygen, pH, temperature, hydraulic retention time).[2,7,9–13,15–20]

Nitrification

The characteristics of the investigated landfill leachate were high concentrations of ammonium nitrogen (NH_4^+-N) and hardly or non-biodegradable organic compounds. Ammonium is toxic to aquatic species and causes eutrophication in natural water environments and therefore must be eliminated from wastewater.[14]

In this investigation, nitrification was achieved during 14 hours (Fig. 1). Ammonium nitrogen was oxidized for 10 hours with nitrite and nitrate accumulation. By extended aeration (for the next 4 hours), the nitrite completely oxidized into nitrate (Fig. 1).
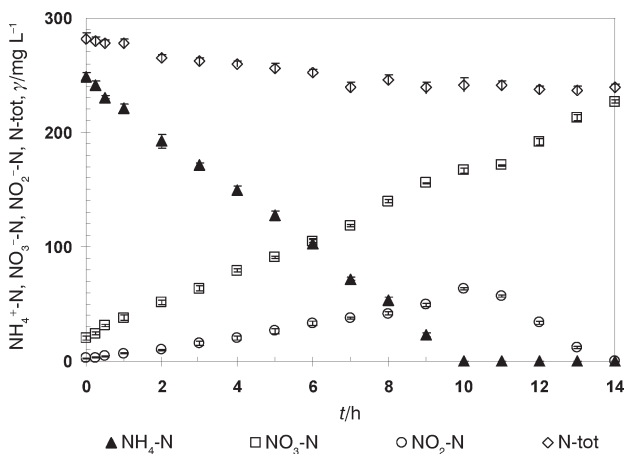

F i g . 1 – *Variations of ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and total nitrogen (N-tot) during nitrification*

Nitrification of landfill leachate is described as a zero-order chemical reaction.[12,14,19] The ammonium nitrogen removal rate and the nitrification rate were calculated by differential equation (1).

$$q_N = \pm \, d\gamma_N/dt = \pm \, k_N \qquad (1)$$

Where:

- q_N – ammonium nitrogen removal rate or nitrification rate, mg NH_4^+-N L^{-1} h^{-1}, mg NO_3^--N L^{-1} h^{-1}
- γ_N – mass concentration of ammonium nitrogen or nitrate nitrogen at time t, mg NH_4^+-N L^{-1}, mg NO_3^--N L^{-1}
- k_N – ammonium nitrogen removal rate constant or nitrification rate constant, mg NH_4^+-N L^{-1} h^{-1}, mg NO_3^--N L^{-1} h^{-1}
- t – time, h

In the given equation, the sign (–) means a decrease in the ammonium nitrogen concentration and sign (+) means an increase in the nitrate nitrogen concentration.

Eq. (1) was solved and experimental data were fitted to the eq. (2):

$$\gamma_N = \gamma_{0,N} \pm k_N \cdot t \qquad (2)$$

- $\gamma_{0,N}$ – initial mass concentration of ammonium nitrogen or nitrate nitrogen, mg NH_4^+-N L^{-1}, mg NO_3^--N L^{-1}

The rate of ammonium nitrogen removal was 24.5 mg NH_4^+-N L^{-1} h^{-1} (Fig. 1). The highest nitrite concentration (63 mg NO_2^--N L^{-1}) was measured in the 10th hour of nitrification, when all ammonium nitrogen was converted to NO_x^--N (NO_2^--N + NO_3^--N) and pH decreased (Fig. 2) to the lowest point, known as the "ammonium valley".[15] This ammonium valley on the pH-profile could be used to indicate the end of nitritation – nitrite production.[15] During nitrification, the COD value remained unchanged (Fig. 2).

In the nitrification experiments, the total nitrogen loss was 14.5 % (Fig. 1). Literature reports that part of the nitrogen is used for biosynthesis, stripping and non-balanced nitrogen.[3,12] The reported values for nitrogen loss during mature landfill leachate nitrification were 10–14 %.[10,12]
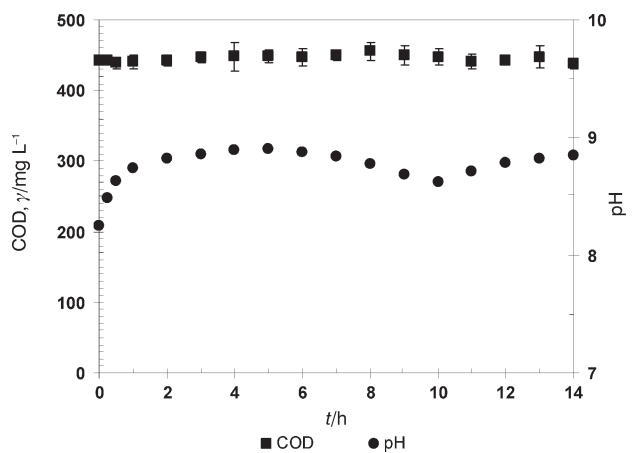

F i g . 2 – *Chemical oxygen demand (COD) and pH during nitrification*

During the additional 4 hours of aeration, the nitrite oxidized into nitrate, and pH began to increase (Fig. 2). After complete nitrification (in the 14th hour), the concentration of NO_3^--N was 228 mg L^{-1} (Fig. 1).

The reason for nitrite accumulation during nitrification could be the inhibition of nitrite oxidation with compounds present in the landfill leachate or some environmental conditions. High initial concentrations of ammonia nitrogen or nitrous acid inhibit nitrite oxidation.[14] High free ammonia (FA) concentrations inhibit not only nitrite oxidizing bacteria but also ammonia oxidizing bacteria. FA concentration is affected by wastewater pH and temperature.[14,15]

Nitrite-oxidizing bacteria are sensitive to FA in the range of 0.1 to 1.0 mg L^{-1}, whereas ammonia-oxidizing bacteria can tolerate FA in the range of 10–150 mg L^{-1}. As a result, nitrite or even ammonium nitrogen can accumulate if the FA level in a reactor is high.[14,15] Concentration of FA in this investigation was calculated according to eq. (3).[14] The results are presented in Fig. 3.

$$\gamma_{FA} = \frac{\gamma_{NH_4-N} \cdot 10^{pH}}{e^{(6334/(273+\vartheta))} + 10^{pH}} \qquad (3)$$

γ_{FA} – mass concentration of free ammonia, mg FA L^{-1}

γ_{NH_4-N} – mass concentration of ammonium nitrogen, mg NH_4^+-N L^{-1}

Fig. 3 shows that FA was gradually reduced from 21.72 mg L^{-1} at the beginning of the experiments (with its maximum value of 46.14 mg L^{-1} in the first hour) to 0 mg L^{-1} during 10 hours of the process.

Although a high FA concentration was present during ammonium removal, ammonium oxidation was performed at the same rate throughout the process. Also, the nitrate production rate remained unaffected.
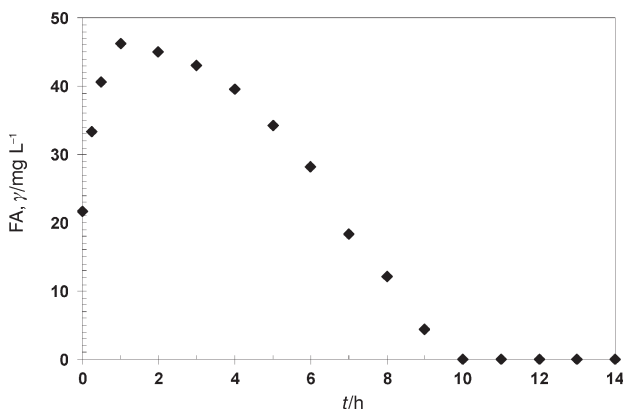

F i g . 3 – *Free ammonia (FA) profile during nitrification*

Some researchers[10,12,15,19] also observed nitrite accumulation during landfill leachate nitrification, while others[11] reported no significant nitrite accumulation. In these investigations, the reasons for nitrite accumulation were: specific organic compounds present in landfill leachate, FNA, FA, landfill leachate composition, low applied HRT, low DO and biomass characteristics.

In the presented study for the determination of nitrification rate, the mass concentrations of nitrate and nitrite nitrogen (NO_x^--N) accumulated during nitrification are summarized and presented in Fig. 4.

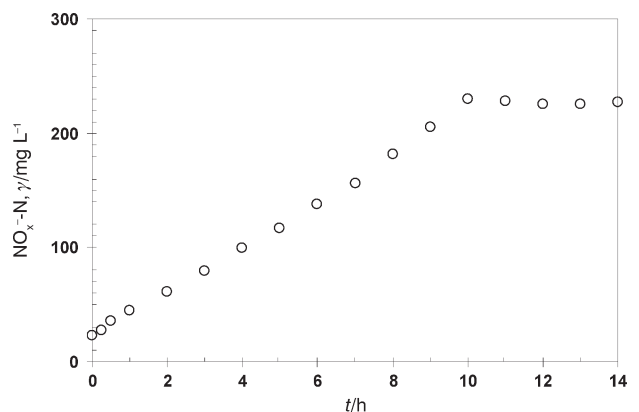F i g . 4 – *Variations of nitrogen oxides (NO_x^--N is expressed as the sum of NO_2^--N and NO_3^--N) during nitrification*

The nitrification rate was calculated according to eq. (1) for the period when NO_x^--N was linear with time, and expressed as mg NO_x^--N L^{-1} h^{-1}. The nitrification rate was 20.1 mg NO_x^--N L^{-1} h^{-1} (Fig. 4).

Literature data about ammonium removal rates from mature landfill leachate were 246 mg NH_4^+-N L^{-1} h^{-1}, 102.8 mg NH_4^+-N L^{-1} h^{-1}, 52–56 mg NH_4^+-N L^{-1} h^{-1}, 20.1 mg NH_4^+-N L^{-1} h^{-1}.[10–12,19] These different values reported depend on landfill leachate characteristics, biomass composition, biomass adaptation, type of reactor, process parameters, N/COD ratio.

In the control experiments (data not shown) 43 % of ammonium nitrogen was lost in 24 hours. There was no nitrite or nitrate accumulation, which is clear evidence that ammonium nitrogen was probably air-stripped. During the experiment, pH increased from 8.24 to 9.24. There was no COD removal. That is in agreement with literature data, which reports that by aeration-air stripping (of mature landfill leachate with initial 140 mg NH_4^+-N L^{-1}) 44 % of NH_4^+-N was reduced after 24 hours of the process, at a temperature of 20 °C and initial pH value of 7.3–7.5, which increased to 9.2 during aeration. Also, ammonia stripping did not begin until pH reached 9.[3]

Denitrification

The investigated landfill leachate had a low COD/N ratio (1.5–1.9) which was why it was an inappropriate source of organic compounds for the process of denitrification. The low COD/N ratio is a characteristic of a mature leachate.[1,6,9] The COD in mature landfill leachate was almost non-biodegradable. Treating of leachate characterized by a low level of biodegradable organic compounds requires a supplementary source of organic carbon to ensure adequate denitrification.

In our experiments, denitrification of nitrified landfill leachate was performed with the addition of sodium acetate as an external carbon source. Sodium acetate dosage per nitrate mass concentration at the beginning of denitrification was 4.95 and 9.9 mg COD per mg NO_3^--N or expressed as C/N ratio 2 and 4, respectively (Fig. 5).
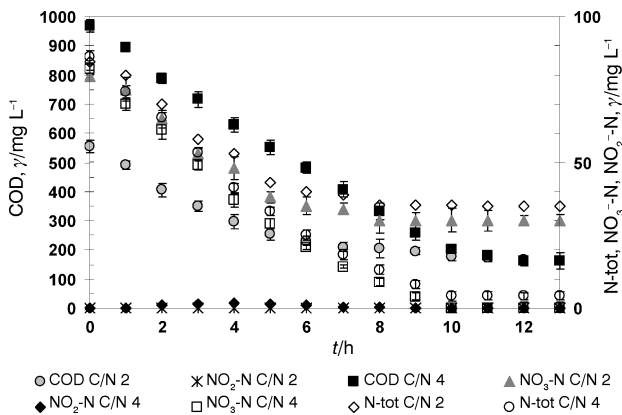

F i g . 5 − *Variations of chemical oxygen demand (COD), nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) during denitrification with sodium acetate in C/N ratio 2 and 4*

Denitrification of the landfill leachate was described as a zero-order chemical reaction[10–12,19] and was expressed as dependent on the nitrate nitrogen concentration versus time according to eq. (4).

$$q_{NO_3-N} = - \, \mathrm{d}\gamma_{NO_3-N}/\mathrm{d}t = - \, k_D \qquad (4)$$

Where:

q_{NO_3-N} – denitrification rate, mg NO_3^--N L^{-1} h^{-1}

k_D – denitrification rate constant, mg NO_3^--N L^{-1} h^{-1}

γ_{NO_3-N} – nitrate nitrogen mass concentrations after time t, mg NO_3^--N L^{-1}

t – time, h

The efficiency of the denitrification process was calculated according to eq. (5).

$$E = \frac{\gamma_0 - \gamma_t}{\gamma_0} \cdot 100 \qquad (5)$$

Where:

E – efficiency of the process, %

γ_0 – mass concentration of compound at the beginning of the process, mg L^{-1}

γ_t – mass concentration of compound at the end of the process, mg L^{-1}

Sodium acetate in dosage of 4.95 mg COD mg^{-1} NO_3^--N (C/N 2) was sufficient for only 62 % of denitrification with remained NO_3^--N in concentration of 30.2 mg L^{-1} (Fig. 5). Denitrification with the addition of sodium acetate at C/N ratio of 4 (9.9 mg COD mg^{-1} NO_3^--N) was performed for 10 hours with no nitrate or nitrite detected at the end of the experiment (Fig. 5). The COD remaining after denitrification processes represents COD from landfill leachate (non-biodegradable COD). The nitrate reduction rate of 8.3 mg NO_3^--N L^{-1} h^{-1} was achieved with C/N ratio of 4 (9.9 mg COD mg^{-1} NO_3^--N). During the denitrification process, total nitrogen decreased simultaneously with nitrate reduction until biodegradable COD was available (Fig. 5).

Denitrification rates obtained from the literature[10,19] were 16.25 mg N L^{-1} h^{-1} and 54 mg N L^{-1} h^{-1} by using sodium acetate as carbon source.

During denitrification of landfill leachate, nitrite accumulation was frequently observed.[2,10,12,17–19] In this research, nitrite accumulation was insignificant. Nitrite concentrations were below 2 mg L^{-1} during denitrification and 0 mg L^{-1} at the end of experiment (Fig. 5).

The literature data reported that nitrate reduction, and nitrite production and reduction during denitrification depend on the dosage of COD per mg NO_3^--N.[2,12,18,20] Different data are available from literature about COD/N ratio required for denitrification of nitrified landfill leachate. According to some authors, the COD/NO_3^--N ratio for complete nitrate reduction in nitrified landfill leachate denitrification, corresponds to 4.9, 5.9 and 7.14.[10,16,19]

In our research, the dosage of sodium acetate was higher than reported in literature. This is not surprising because denitrification not only depends on carbon source used but also on species composition, nitrite and environmental parameters.[2] In our investigation a denitrification profile is a characteristic of culture composition and their origin from landfill leachate where the microorganisms were continuously exposed to landfill leachate composition, which probably ensured adequate microbial species composition. Also, other authors reported about community composition as an important factor for the stability of the landfill leachate treatment process.[2,4,10–13,20]

Conclusions

The microbial culture originating from leachate has shown ability and activity for processes of nitrification and denitrification of mature landfill leachate. The ammonium oxidation rate was 24.5 mg NH_4^+-N L^{-1} h^{-1}. During nitrification, nitrate and nitrite were accumulated (nitrite accumulation rate was 5.6 mg NO_2^--N L^{-1} h^{-1} and nitrate accumulation rate was 14.4 mg NO_3^--N L^{-1} h^{-1}). During extended aeration, the nitrite completely oxidized into nitrate. The nitrification rate expressed as the sum of mass concentrations of nitrate and nitrite nitrogen was 20.1 mg NO_x^--N L^{-1} h^{-1}. COD in landfill leachate was non-biodegradable. Denitrification was studied with the addition of sodium acetate as a carbon source at COD/N ratio 4.95 and 9.9 mg COD per mg NO_3^--N. Complete denitrification was performed at COD/N ratio of 9.9 mg COD per mg NO_3^--N with denitrification rate of 8.3 mg NO_3^--N L^{-1} h^{-1}.

List of abbreviations and symbols

BTEX – Benzene, Toluene, Xylene, mg L^{-1}

BOD – Biochemical Oxygen Demand, mg L^{-1}

COD – Chemical Oxygen Demand, mg L^{-1}

DO – Dissolved Oxygen, mg L^{-1}

FA – Free Ammonia, mg L^{-1}

FNA – Free Nitrous Acid, mg L^{-1}

HRT – Hydraulic Retention Time, days

NH_4^+-N – Ammonium nitrogen, mg L^{-1}

NH_3 – Ammonia, mg L^{-1}

NO_3^--N – Nitrate nitrogen, mg L^{-1}

NO_2^--N – Nitrite nitrogen, mg L^{-1}

N-tot – Total nitrogen, mg L^{-1}

PO_4-P – Phosphorous orthophosphate, mg L^{-1}

PAH – Polycyclic Aromatic Hydrocarbons, mg L^{-1}

PCB – Polychlorinated biphenyls, mg L^{-1}

TOC – Total Organic Carbon, mg L^{-1}

XOCs – Xenobiotic Organic Compounds, mg L^{-1}

q_N – ammonium nitrogen removal rate or nitrification rate, mg NH_4^+-N $L^{-1}h^{-1}$, mg NO_3^--N L^{-1} h^{-1}

$q_{NO_3\text{-}N}$ – denitrification rate, mg NO_3^--N L^{-1} h^{-1}

γ_N – mass concentration of ammonium nitrogen or nitrate nitrogen at time t, mg NH_4^+-N L^{-1}, mg NO_3^--N L^{-1}

γ_{FA} – mass concentration of free ammonia, mg FA L^{-1}

$\gamma_{NH_4\text{-}N}$ – mass concentration of ammonium nitrogen, mg NH_4^+-N L^{-1}

$\gamma_{NO_3\text{-}N}$ – nitrate nitrogen mass concentrations after time t, mg NO_3^--N L^{-1}

$\gamma_{0,N}$ – initial mass concentration of ammonium nitrogen or nitrate nitrogen, mg NH_4^+-N L^{-1}, mg NO_3^--N L^{-1}

$\gamma_{NOx\text{-}N}$ – sum of nitrite and nitrate nitrogen mass concentrations after time t, mg NO_x^--N L^{-1}

k_N – ammonium nitrogen removal rate constant or nitrification rate constant, mg NH_4^+-N L^{-1} h^{-1}, mg NO_3^--N L^{-1} h^{-1}

k_D – denitrification rate constant, mg NO_x^--N L^{-1} h^{-1}

t – time, h

ϑ – temperature, °C

E – process efficiency, %

γ_0 – mass concentration of compound at the beginning of the process, mg L^{-1}

γ_t – mass concentration of compound at the end of the process, mg L^{-1}

References

1. *Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A., Christensen, T. H.,* Crit. Rev. Environ. Sci. Tech. **32** (2002) 297.
2. *Martienssen, M., Schöps, R.,* Wat. Res. **31** (1997) 1164.
3. *Marttinen, S. K., Kettunen, R. H., Sormunen, K. M., Soimasuo, R. M., Rintala, J. A.,* Chemosphere **46** (2002) 851.
4. *Černila Zajc, N., Glancer, M., Grömping, M., Šoljan, V.,* Chem. Biochem. Eng. Q. **18** (2004) 77.
5. *Wiszniowski, J., Robert, D., Surmacz-Gorska, J., Miksch, K., Weber, J. V.,* Environ. Chem. Lett. **4** (2006) 51.
6. *Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F., Moulin, P.,* J. Hazard. Mater. **150** (2008) 468.
7. *Salem, Z., Hamouri, K., Djemaa, R., Allia, K.,* Desalination **220** (2008) 108.
8. *Xu, Y., Zhou, Y., Wang, D., Chen, S., Liu, J., Wang., Z.,* J. Environ. Sci. **20** (2008) 1281.
9. *Kulikowska, D., Klimiuk, E.,* Bioresource Technol. **99** (2008) 5981.
10. *Doyle, J., Watts, S., Solley, D., Keller, J.,* Water Sci. Technol. **43** (2001) 315.
11. *Yalmaz, G., Öztürk, I.,* Water Sci. Technol. **43** (2001) 307.
12. *Kulikowska, D., Klimiuk, E.,* Polish J. Environ. Stud. **13** (2004) 389.
13. *Klimiuk, E., Kulikowska, D.,* Polish J. Environ. Stud. **5** (2004) 525.
14. *Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., Srinatu, E. G.,* J. Water Pollut. Control Fed. **48** (1976) 835.
15. *Peng, Y., Zhang, S., Zeng, W., Zheng, S., Mino, T., Satoh, H.,* Wat. Res. **42** (2008) 883.
16. *Carley, B. N., Mavinic, D. S.,* Res. J. Water Pollut. Control Fed. **63** (1991) 51.
17. *Manoharan, R., Liptak, S., Parkinson, P., Mavinic, D., Randall, C. W.,* Environ. Technol. **10** (1989) 707.
18. *Welander, U., Henrysson, T., Welander, T.,* Wat. Res. **32** (1998) 1564.
19. *Kaczorek, K., Ledakowicz, S.,* Bioprocess Biosyst. Eng. **29** (2006) 291.
20. *Loukidou, M. X., Zouboulis, A. I.,* Environ. Pollut. **111** (2001) 273.
21. APHA, AWWA and WEF, Standard methods for the examination of water and wastewater, United Book Press, Baltimore, 1998.
22. Pravilnik o graničnim vrijednostima opasnih i drugih tvari u otpadnim vodama. NN RH 94, 2008.