sciendo

Modelling in civil and environmental engineering
Vol. 16-No. 4 : 34-44-2021
Doi: 10.2478/mmce-2021-0019

BIOLOGICAL DENITRIFICATION OF WATER INTENDED FOR HUMAN CONSUMPTION

Alexandru MATEI – Ph.D. Student, Technical University of Civil Engineering Bucharest, Faculty of Hydrotechnics, e-mail: mateialexandru.ioan@yahoo.com

Gabriel RACOVIŢEANU - Prof.dr.eng., Technical University of Civil Engineering Bucharest, Faculty of Hydrotechnics

Elena VULPAŞU - Conf.dr.chem.eng., Technical University of Civil Engineering Bucharest, Faculty of Hydrotechnics

Abstract: Nitrate is the most common form of nitrogen in water. The presence of nitrates in drinking water can harm human health, this is why the maximum concentration of nitrates in potable water is imposed by law. The main treatment technologies used for treating this kind of water are reverse osmosis, ion exchangers, electrodialysis and biological denitrification. The goal of this paper is to determine the efficiency of biofilters in reducing the ammonium concentrations in water, depending on the carrier/filtration media used in the biofilters, the C:N:P ratio used and contact time between water and the attached biomass. The paper is based on the result obtained during experimental tests in which the concentration of nitrates in the raw water was 150 mg/l.

Keywords: Biofilter, Nitrates, Active Carbon, Sand, Ceramic granules, Biomass

1. Introduction

The nitrate ion is a chemical compound made up of a nitrogen atom bound to three oxygen atoms and which is designated by the symbol "NO_3^-". This is the most common form of nitrogen in water and also, the main source of nitrogen for plants, being at the same time a nutrient without which they cannot grow.

The main cause of nitrogen pollution is considered to be intensive agriculture. Other causes as well as discharges of insufficiently treated or untreated wastewater and a lack of a centralized system for collecting and treating domestic wastewater in localities where there are centralized drinking water distribution systems.

The concentration of nitrates in surface waters has increased in recent decades, in some areas, doubling in the last 20 years. In the United Kingdom, there is an increase in the concentration of nitrates of about 0.7 mg / l for some rivers [1]

In the case of groundwater, the natural concentration of nitrates is a few milligrams per liter, but this is strongly influenced by the type of soil and the geological situation. As a result of agricultural activities, the concentration of nitrates in groundwater can increase dramatically, reaching values of hundreds of milligrams per liter. In groundwater in agricultural areas in India, concentrations of 1500 mg / l of nitrate have been recorded [2].

An increase in nitrate concentrations is observed worldwide for groundwater.

In 1970, the World Health Organization imposed a maximum allowable concentration of 100 mg/l, the recommended concentration of nitrates being below 50 mg/l. The guideline level according to the World Health Organization for Nitrates has remained the same today - 50 mg/l [3] [4] [5].

The maximum concentration of nitrates in drinking water required by Directive 2184/2020 / EU is 50 mg/l.

In Romania, the maximum permissible limit for nitrates in drinking water, according to law 458/2002, is 50 mg/l [5]

According to the EPA in the United States, the maximum allowable limit for nitrates in drinking water is 10 mg N/l [6]

The lethal dose of nitrates for humans varies between 67 and 833 mg/kg body weight and 33 to 250 mg/kg body weight for nitrites [1].

The presence of nitrates in drinking water can have negative effects on human health. Nitrates can turn into nitrites, affecting children under three months old and inducing methemoglobinemia. Nitrates can also form compounds such as nitrosamines and nitrosamides, compounds with carcinogenic potential [1].

The lethal dose of nitrates for humans ranges from 67 to 833 mg/kg and 33 to 250 mg/kg for nitrites [1].

Most of the time, the use of an alternative source of water is not possible, imposing treatment of water before distribution. Over time, several methods have been developed to reduce the concentration of nitrates in water intended for human consumption:

- Biological processes;
- Reverse osmosis;
- Electrodialysis;
- Ion exchangers.

2. Biological processes

The use of biological processes has been introduced relatively recently in water treatment for water with high nitrates concentrations.

Biological denitrification is the biological process of reducing nitrates to gaseous nitrogen with the help of denitrifying bacteria. The process of biological denitrification has the great advantage that as a result, nitrate is transformed into gaseous nitrogen, which has no harmful effects on the environment or human health [6] [7].

This process is performed in several stages, the nitrate being successively reduced to nitrogen compounds according to the following relation.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \text{ [3]} \quad (1)$$

In most cases, the process of converting nitrate to nitrogen gas is considered a two-step process, namely, the conversion of nitrate to nitrite ($NO_3^- \rightarrow NO_2^-$) and the transformation of nitrite into nitrogen gas ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$).

Each step of the denitrification process can be written, representing the equivalent electrons transferred from the organic substrate (e^-).

$$2e^- + NO_3^- + 2H^+ \rightarrow NO_2^- + H_2O \quad (2)$$

$$e^- + NO_2^- + 2H^+ \rightarrow NO + H_2O \quad (3)$$

$$2e^- + 2NO + 2H^+ \rightarrow N_2O + H_2O \quad (4)$$

$$2e^- + N_2O + 2H^+ \rightarrow N_2 + H_2O \quad (5)$$

Combining the above equations, we obtain:

$$10e^- + 2NO_3^- + 12H^+ \rightarrow N_2 + 6H_2O \quad (6)$$

Or the equivalent

$$0.2NO_3^- + 1.2H^+ + e^- \rightarrow 0.1N_2 + 0.6H_2O \quad (7)$$

Similarly, the oxygen reduction equation is:

$$0.25O_2 + H^+ + e^- \rightarrow 0.5H_2O \quad (8)$$

It follows that 0.25 moles of oxygen are the equivalent of 0.2 moles of nitrate in the case of the transfer of an electron in the oxidation reaction [8].

Because the oxygen needed in bacterial respiration is supplied by nitrate, the denitrification process is designed to occur in the absence of oxygen (anoxic conditions).

The denitrification process is influenced by certain inhibitory factors such as temperature, dissolved oxygen concentration, pH and alkalinity, inhibitors and the ratio between electron donor and nitrogen [7].

Advantages of biological denitrification

The main advantages of using biological processes are:

- Low costs, compared to other technologies used (reverse osmosis, ion exchangers, etc.);
- Good efficiency, regardless of the amount of nitrates from the raw water;
- The process does not need chemical substances addition.

Disadvantages of biological denitrification

The main disadvantages of the biological denitrification technique are:

- The amount of time required for the startup of the biological process is larger than all the other available technologies;
- Additional processes are needed to eliminate substances resulting from microbial activity;
- For a proper operation, careful maintenance and monitoring of biomass and its composition are required.

2.1. External carbon source

Based on utilized food and energy sources, bacteria are classified into two main categories, named „heterotrophic" and „autotrophic".

Heterotrophic denitrifying bacteria require a source of organic carbon for respiration. If sufficient amounts of organic carbon are not dissolved in water, added substances such as methanol, ethanol, etc. may be used [7].

Autotrophic denitrifying bacteria are bacteria that do not require carbon sources for respiration and therefore produce denitrification by themselves. They use hydrogen or reduced sulfur species as a substrate and carbon dioxide or bicarbonate is used as the carbon source [1].

The rate of denitrification and multiplication of bacteria is influenced by the amount and type of carbon source.

The main external sources of carbon used are acetic acid, methanol, ethanol and glucose.

It has been observed that the most effective substrate is acetic acid, followed by ethanol and methanol [8].

Methanol carries the risk of the potential toxicity of residual methanol in treated water [11]. 4-15 ml of methanol can cause blindness, and a dose of more than 300 mg/kg body weight can be fatal [9]. To eliminate the problems caused by the toxicity of methanol as a carbon source in nitrogen removal, ethanol is used as a safe alternative carbon source. No limit has been set for ethanol in drinking water [10].

The stoichiometric reactions for the use of methanol, ethanol, glucose and acetate are as follows

Methanol [11]

$$5CH_3OH+6NO_3^- \rightarrow 3N_2 +5CO_2+H_2O+6OH \quad (9)$$

Ethanol [8]

$$5CH_3CH_2OH +12NO_3^- \rightarrow 6N_2 +10CO_2 + 9H_2O +12OH \quad (10)$$

Glucose [8]

$$5C_6H_{12}O_6 + 24NO_3^- \rightarrow 12N_2 + 30CO_2 + 18H_2O + 24OH \quad (11)$$

Acetic acid [11]

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad (12)$$

2.1 The biofilm

All microorganisms can form biofilms. It can develop in days or months, depending on the intake of organic substances. The biofilm uses a solid medium as a support layer, forming EPS-Extracellular Polymeric Substances [3] [12].

The most important processes that occur in the activity of a biofilm are the fixation of microorganisms, the development of biomass and the detachment of the biofilm from the support.

Biomass fixation defines the adhesion of microorganisms to the support state.

The development of biomass requires the contribution of organic components and substrate for feeding microorganisms.

The main factors that influence the use of the substrate in a biofilm are:

- Transport of the substrate mass to the biofilm;
- Substrate diffusion in biofilm;
- Biofilm kinetics.

The relationships that govern the phenomena that take place in biofilm during the development stage are presented below [3].

The transport of the mass of the substrate to the biofilm is given by Fick's first law [3].

$$J = \frac{AD(S - S_S)}{L_d} \quad (13)$$

Where:

J - substrate flow to biofilm [mg/m^2/s];

A - surface area of the support medium [m^2];

D - molecular diffusion coefficient of the substrate in the whole mass of the liquid [m2/s];

S - concentration of substrate in the mass of solution [mg/l];

S_S - substrate concentration on the outside of the biological film [mg/l];

L_d - the thickness of the diffusion layer [m].

The transport of the substance into the biofilm by molecular diffusion is given by Fick's second relation [3].

$$r_d = D_f \frac{\partial^2 S_b}{\partial Z^2} \quad (14)$$

Where:

r_d - the degree of substrate accumulation due to diffusion [mg/m^3/s];

D_f - molecular diffusion coefficient from biofilm [m^2/s];

S_b - biofilm substrate concentration [mg/l];

Z - distance to the surface of the biofilm [m].

The substrate usage relationship is given by Monod's relation [3].

$$r_u = \frac{kX_f S_b}{K_S + S_b} \quad (15)$$

Where:

r_u - the degree of use of biofilm substrate [mg/m^3/s];

k - maximum specific degree of substrate use [mg substrate/mg biomass/s];

X_f - biomass density of biological film [mg/l];

K_s - Monod coefficient [mg/l];

The rate of development of biomass in conditions of instability can be calculated using the following relation [3].

$$r_g = \frac{YkX_f S_b}{K_S + S_b} A_f L_f \quad (16)$$

Where:

r_g - the rate of biomass development in the biofilm [mg/s];

Y - yield [mg / mg];

k - maximum specific degree of substrate use [mg substrate/mg biomass/s];

X_f - biomass density of biological film [mg/l];

A_f - The surface area of the biofilm [m^2];

L_f- biological film thickness [m];

S_b - biofilm substrate concentration [mg/l];

K_s - Monod coefficient [mg/l];

Detachment of biomass from the substrate is one of the most important mechanisms that influence the efficiency of the biological process. The most common mechanisms that lead to biomass detachment are erosion, exfoliation and abrasion.

3. Material and methods

3.1 Scope of the experimental tests

Experimental research was performed on a pilot biological denitrification installation. The main objectives of the experimental study were:

- Testing the efficiency of biological denitrification for three types of filter media used: activated carbon granules, ceramic granules and sand;
- Estimation of the active height of the filter layer in the biofilter;
- Studying the behavior of biomass at a low amount of carbon in raw water and low retention time;
- Estimation of a nitrogen reduction capacity for developed biomass.

3.2 Experimental installation

The experimental tests were performed on an experimental stand that is part of the Technical University of Civil Engineering of Bucharest laboratories.

The experimental stand is represented by a pilot installation, consisting of 3 biofilters with fixed bed and descending flow. The height of the filtration bed is 1 m for all biofilters.

The diagram of the pilot plant is shown in the following figure.

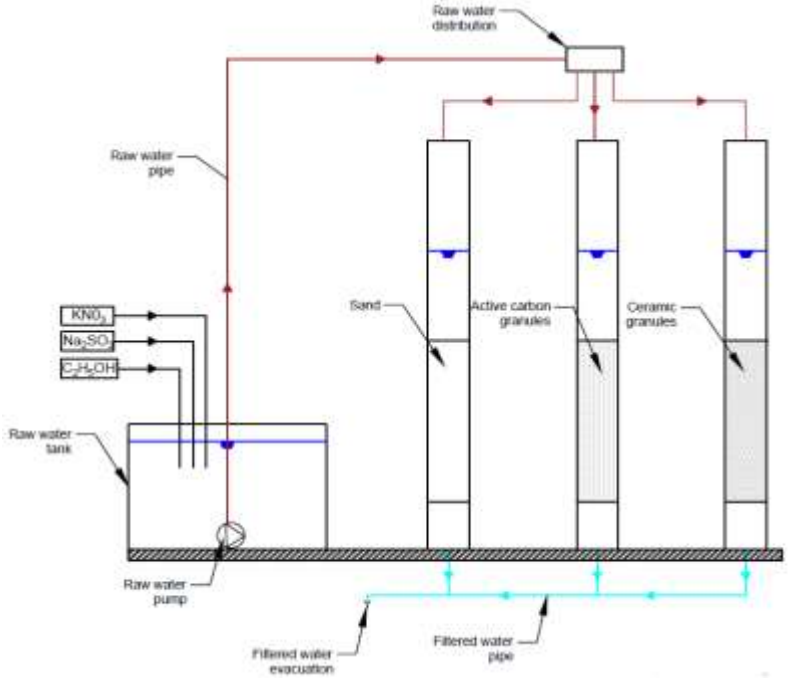


Fig. 1 – The pilot installation.

3.3 Experimental protocol

The experimental tests lasted for three weeks, one week for every cycle. The samples were taken during the weekend.

For every cycle, the following steps were followed:

- After completion of biomass development/finishing of an experimental cycle, a solution with a concentration approximately equal to 150 mg/ was used as raw water. The plant operated by recirculating the biofilter effluent for three days.
- After 3 days, the water in the plant was replaced with a new solution with the same concentration of nitrates and the plant continued to operate using effluent recirculation.
- The day before the experimental determinations, the water in the installation was replaced again and the installation operated without recirculating the flow until the next day.
- On the day of the test, the raw water was replaced again and the experimental determinations began, taking water samples from both the effluent of each biofilter and their filter layers.
- At the end of the experimental cycle, the water in the installation was replaced with a solution that had the characteristics used in the next experimental cycle.
- The steps were repeated for each experimental cycle.

3.4 Raw water

The parameters considered for each experimental cycle are presented in the following table.

Synthetic raw water parameters [13]

Experimental test	Nitrates (mg/l)	C:N:P ratio	Flow rate (m/h)
First cycle	150	13:01	2.54
Second cycle	150	1.3:1	2.54
Third cycle	150	13:01	5.08

The following table shows the quantities of substances needed for raw water preparation. Sodium sulfite is used to keep the dissolved oxygen concentration in water below 0.2 mg / l. The stoichiometric ratio is 10 mg sulfite for 1 mg of oxygen, a value taken from the literature [14] [15] [16].

Chemical substances used for raw water preparation [13]

Experimental test	Water volume (l)	Nitrates (mg/l)	C:N:P ratio	KNO3 (g)	Phosphoric acid (ml)	Alcohol 96% (ml)	Sodium sulfide (g)
First cycle	1000	150	01:00.2	245	14	282.5	280
Second cycle	1000	150	1.3:1:0.2	245	14	28	300
Third cycle	1000	150	01:00.2	245	14	282.5	300

4. Results and Discussions

4.1 Biofilters efficiency

The following table shows the results obtained in the experimental test for biological denitrification.

Biological denitrification – results obtained [13]

Experimental cycle	Nitrates (mg/l)				Efficiency (%)		
	Raw water	Filtrate					
		Active carbon granules	Ceramic granules	Sand	Active carbon granules	Ceramic granules	Sand
1	150	2.13	1.07	7.83	98.58%	99.29%	94.78%
2	150	0.24	54.1	77.1	99.84%	63.93%	48.60%
3	150	UDL*	0.387	UDL*	99.99%	99.74%	99.99%
Minimum efficiency (%)					98.58%	63.93%	48.60%
Medium efficiency (%)					99.47%	87.65%	81.12%
Maximum efficiency (%)					99.99%	99.74%	99.99%

UDL* - Under detection limit

For all the concentrations of nitrates in the raw water, under a C:N:P ratio of 13:1:0.2, the biofilters reduced the nitrate concentration in the water to values below the 50 mg/l limit for all analyzed samples.

In the case of reducing the carbon dose from the external source by 90%, the concentration of nitrates was below the limits imposed by law only for the water treated in the activated carbon biofilter, which has a very high reduction efficiency of 99.84%. Resulting that the biomass attached to the activated carbon filter being less sensitive than the biomass from the other two filter media analyzed, which have shown a higher sensitivity of the biomass attached to reducing the amount of nutrients.

In the case of halving the retention time in the filter layer, all biofilters reduced the concentration of nitrates in water to a value below the imposed limit (50 mg/l), thus demonstrating the ability of biofilters to reduce the amount of nitrates even at high speeds. filtering. By doubling the filtration rate, a flow rate treated by each filter of 40 l/h was obtained, an amount of nitrates in the raw water of 6.0 g NO_3/h in each biofilter. The concentration of nitrates in the filtered water is close to 0 mg/l, the retention time in the filter layers is 11.8 minutes, resulting in a reduction rate of nitrates of about 150 mg/l/h.

The following graph shows the nitrate concentration reduction efficiency of each biofilter at the end of each experimental cycle. The C:N:P ratio used in the experimental cycle is shown for each filtration cycle.

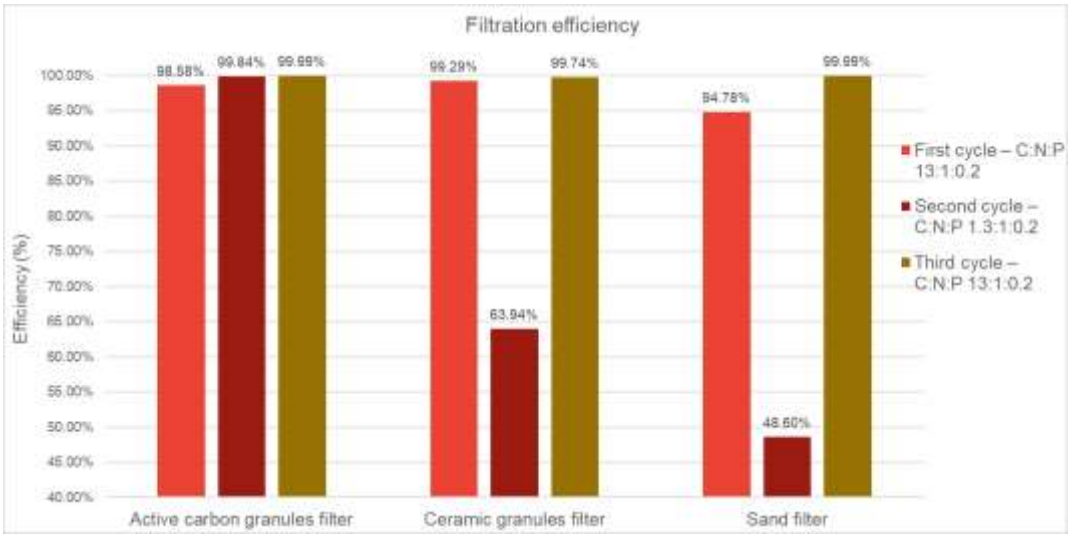


Fig. 2 – Denitrification efficiency [13].

4.2 Reduction of nitrates inside the filter

It is observed that the reduction of the largest amount of nitrates from the raw water occurs in the first third of all biofilters. The values of the nitrate concentration determined for the filtered water taken from the first 30 cm of each filter layer as well as the reduction efficiency for each filter layer are presented in the following table.

Reduction efficiency – first third [13]

Cycle	Raw water	Active carbon granules filter		Ceramic granules filter		Sand filter	
	Nitrates (mg/l)	Nitrates (mg/l)	Efficiency (%)	Nitrates (mg/l)	Efficiency (%)	Nitrates (mg/l)	Efficiency (%)
First cycle	150	14.6	90.27%	10.4	93.07%	13.1	91.27%
Second cycle	150	41.3	72.47%	88	41.33%	80.8	46.13%
Third cycle	150	0.325	99.78%	1.12	99.25%	1.18	99.21%

The following graph shows the nitrate concentration reduction efficiency in the first third of each biofilter at the end of each experimental cycle. The C:N:P ratio used in the experimental cycle is shown for each filtration cycle.

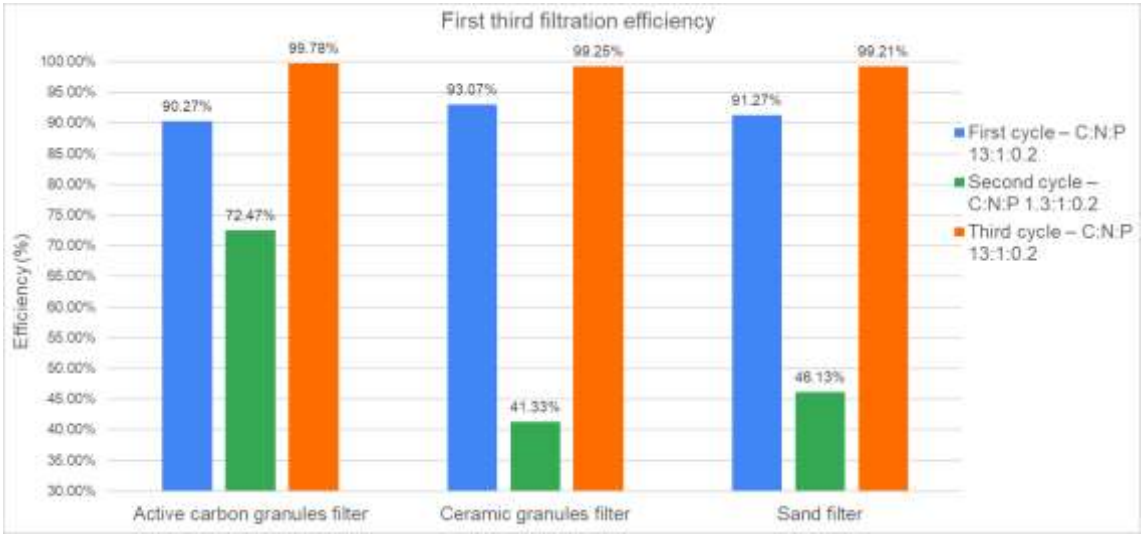


Fig. 3 – Denitrification efficiency – first third [13].

The following figures show the distribution of nitrate concentration in the filter layers of the biofilters for water taken at the end of every filtration cycle. To create the distribution graphs of

the reduction of the concentration of nitrates inside each filter layer, the values determined for the water samples taken from each filter layer, from different heights, were considered.

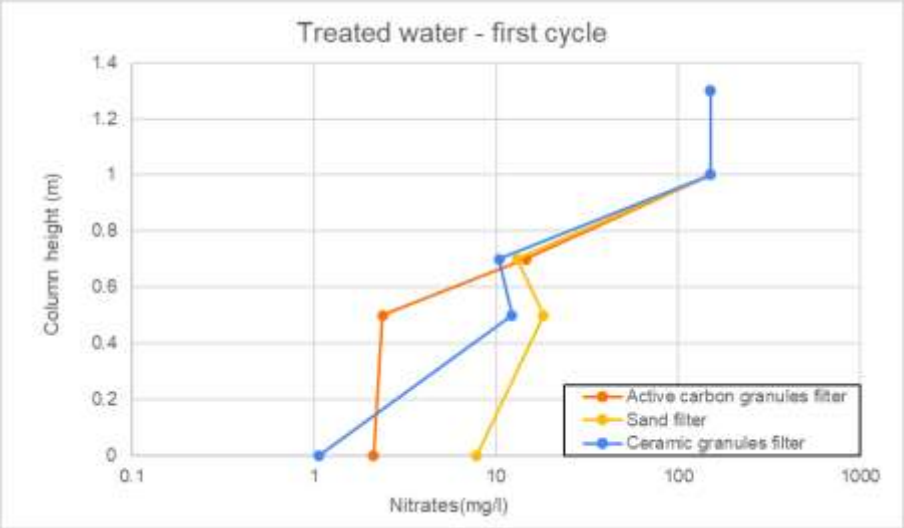


Fig. 4 –Distribution of nitrates inside the biofilters – cycle 1 [13]

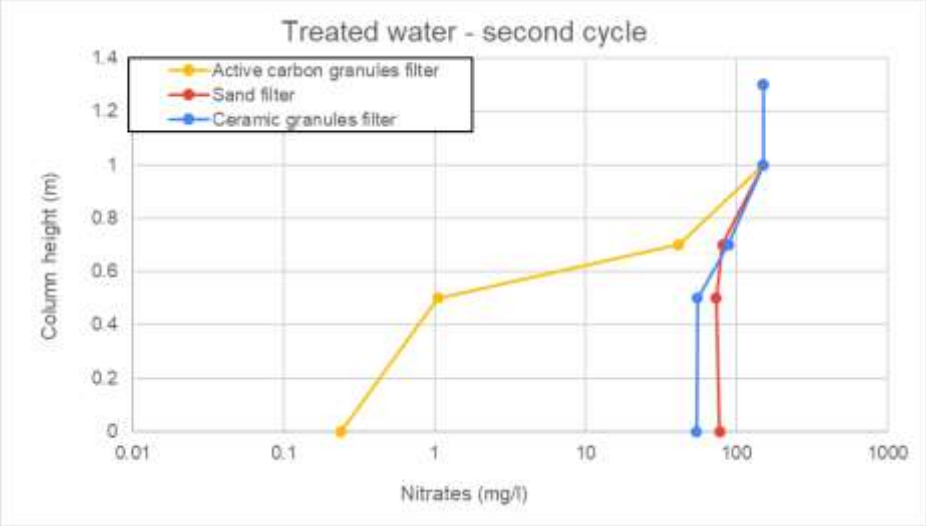


Fig. 5 –Distribution of nitrates inside the biofilters – cycle 2 [13]

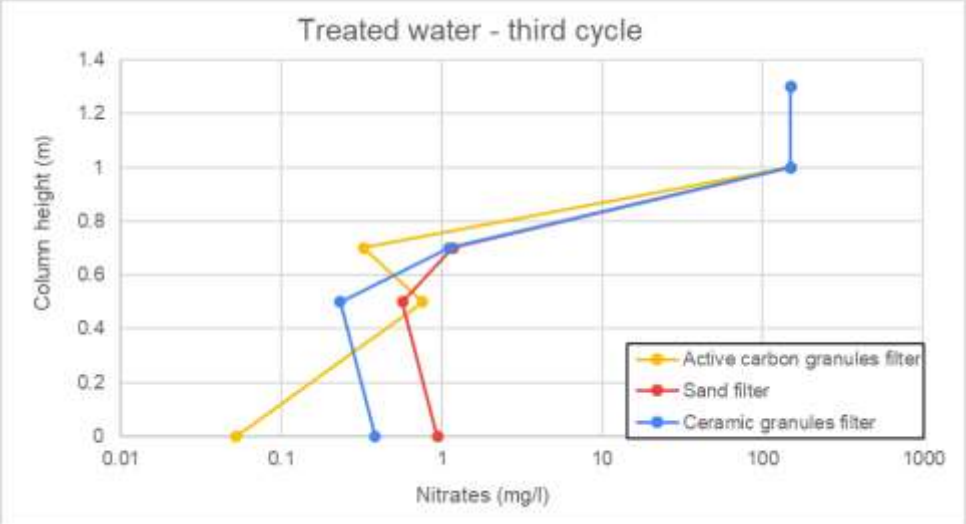


Fig. 6 –Distribution of nitrates inside the biofilters – cycle 3 [13]

The highest amount of nitrates was reduced in the first part of each filter, in the first 30 cm of the filter, the influential amount of nitrate was reduced by 90%. It turns out that in biological filtration, the active height of the filter is about 30% of the total height of the filter.

5. Conclusions

According to the results obtained from the experimental tests, the following can be stated:

- The biological denitrification process worked during the experimental tests, a reduction of nitrates from the raw water was obtained;
- The results show that the biofilter of carbon active granule filtration bed gives the best result, being the only one who could reduce the nitrates value from the raw water under the imposed concentration (50 mg/l);
- Retention time, one of the most important factors in bioreactors design was very short, 23.6 minutes, but the biofilters reduced the quantity of nitrates from the raw water under 50 mg/l even in case of doubling the filtering speed and thus reducing the retention time by half to 11.8 minutes;
- The maximum quantity of nitrates from raw water was reduced in the first third of every biofilter, resulting that the active height of the biofilters was three times smaller than the filter height;
- From the technologies available for reducing the amount of nitrates in water, biological denitrification is an efficient solution that can treat waters with large amounts of nitrates, can take large variations of loading and can prove to be a cheaper and easier to implement solution than commonly used solutions such as ion exchange technologies and reverse osmosis.

References

[1] World Health Organization. (2003) *Nitrate and Nitrite in Drinking-water- Draft background document for development of WHO Guidelines for Drinking-water Quality,* World Health Organization https://apps.who.int/iris/handle/10665/75380

[2] Jacks, G. Sharma, V. P (1983) *Nitrogen circulation and nitrate in groundwater in an agricultural catchment in Southern India,* Environmental Geology, 5, 64-64. DOI: 10.1007/BF0238109

[3] Calin, C. (2011) *Procese si Tehnologii pentru Controlul Continutului de Azot din Apa*. Unpublished doctoral thesis, Technical University of Civil Engineering of Bucharest.

[4] Iacob, O. Tudor, A. Cristea, A. (2012) *Apa de fântână – contaminarea cu azotaţi şi methemoglobinemia,* Universal publishing house Carol Davila, Bucharest

[5] Romanian Government - *„Law no. 458/2002"*

[6] U.S. Environmental Protection Agency (2008) *EPA's Report on the Environmen*t, Washington, DC

[7] Mohseni-Bandpi, A. Elliott, DJ. Zazouli, MA. (2013) *Biological nitrate removal processes from drinking water supply-a review,* Journal of environmental health science & engineering, 11, 35. DOI: 10.1186/2052-336X-11-35

[8] Mokhayer, Y. (2010) *Characterizing denitrification kinetics and stoichiometry",* The Faculty of The School of Engineering and Applied Science, Washington

[9] Moon, C.S. (2017) *Estimations of the lethal and exposure doses for representative methanol symptoms in humans,* Annals of occupational and environmental medicine, 29, 44. DOI: 10.1186/s40557-017-0197-5

[10] Pohanish. P R. (2011) *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens*, USA: Elsevier Inc.

[11] Metcalf. &. Eddy (2003) *Wastewater Engineering: Treatment and Reuse"*, New York, McGraw-Hill.

[12] Rabin, N. Zheng, Y. Opoku-Temeng, C. Du, Y. Bonsu, E. Sintim H.O. (2015) *Biofilm formation mechanisms and targets for developing antibiofilm agents,* Future Medical Chemistry, 7(4), 493-512. DOI: 10.4155/fmc.15.6

[13] Matei, A. (2021) *Cercetari experimentale privind tehnologiile de tratare a apei in vederea reducerii concentratiilor de azotati*. Technical University of Civil Engineering of Bucharest. (No.3)

[14] Cloug, S. (2014) *Encyclopedia of Toxicology (Third Edition),* Academic Press.

[15] Crawford, P.W. (1996) *Catalyzed sodium sulfite for oxygen removal from injection water*, Prod. Mon., 30(1)

[16] L. K. W. Industries (1998) *Oxygen scavenger and boiler water treatment chemical,* Europa Brevet EP 0845438A1, 03 06 1998