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NITRATES REDUCTION FROM WATER INTENDED FOR HUMAN CONSUMPTION THROUGH SPECIAL WATER TREATMENT PROCESSES

-DOCTORAL THESIS-

- SUMMARY-

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Table of contents

1 Introduction. General elements 4
1.1 Nitrogen circuit in nature 4
1.2 Nitrogen in the environment 4
1.2.1 Air 4
1.2.2 Food 4
1.2.3 Water 5
1.3 Effects of nitrogen compounds on the human body 5
1.4 Nitrogen sources in water 6
1.4.1 Surface waters 6
1.4.2 Groundwater 6
1.5 Pollution with nitrogen compounds in Romania 7
1.5.1 Nitrates Directive. The general framework and the situation in Romania 7
2 Technologies used to reduce the concentration of nitrates in drinking water 10
2.1 General elements 10
2.2 Reverse osmosis 10
2.3 Ion exchangers 10
2.4 Electrodialysis 11
2.5 Denitrification of water using biological processes 11
2.6 Biofilters 11
2.6.1 Development of the process 11
2.6.2 The Biofilm 13
2.6.3 Substrate 13
2.6.4 Biofilters operation 14
3 Pilot installation and experimental protocol 15
3.1 Experimental installation 15
3.2 Experimental stages 17
3.2.1 Stage 1 - Development of the denitrifying biomass 18
3.2.2 Stage 2 - Analysis of the efficiency of biofilters 18
3.3 Parameters analyzed 19
4 The results of experimental research 20
5 General conclusions 26
5.1 Elements of originality and personal contributions 27
5.2 Recommendations for future research 28
6 Selective bibliography 29

Table list

Table. 2.1 Biofilters use in water treatment [1]. 12
Table 3.1. Filter media used. 17
Table 3.2. Raw water parameters during experimental cycles. 18
Table 4.1 Results obtained - reduction of nitrate concentrations. 20
Table 4.2. Results obtained - Oxidability of water. 24

Figures list

Figure 3.1. Experimental installation scheme. 16
Figure 3.2. Photos of the experimental installation. 16
Figure 3.3. Filter material - granular activated carbon granules. 17
Figure 3.4. Filter material - filter sand. 17
Figure 3.5. Filter material - ceramic granules. 17
Figure 4.1. Nitrates reduction efficiency. 22
Figure 4.2. Nitrogen reduction efficiency in the first third of the layer. 23

1 Introduction. General elements

Nitrogen compounds in natural, surface or groundwater exist in many forms, depending on their nature and the state of oxidation in which they are found.

The nitrogen 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 the water.

It is the main source of nitrogen for plants, being at the same time a nutrient without which they cannot grow. Intensive agriculture can deplete the soil of nitrogen compound resources, so the addition of nitrogen fertilizers is needed. Properly managed, nitrogen does not endanger human health and increases agricultural production. However, if the amount of nitrate introduced into the soil is higher than the plant needs, the excess can seep into groundwater, contaminating them.

1.1 Nitrogen circuit in nature

Nitrogen is also a vital component for microorganisms. Nitrogen gas (N_2) represents 78% of the atmosphere. But although it is present in nature in very large quantities, it is often found in forms inaccessible to plants and animals.

As it passes through the nitrogen circuit, a nitrogen atom can bind in several chemical, organic and inorganic forms. These transformations involve oxidation or reduction reactions of the nitrogen atom, most of which are microbially mediated [1].

The biological circuit of nitrogen is a slow and continuous process in which nitrogen gas from the atmosphere is converted by over 100 species of bacteria into fixed forms that are used by plants and animals. The organic nitrogen compounds are then mineralized and returned to the atmospheric reservoir [2].

1.2 Nitrogen in the environment

1.2.1 Air

The average annual concentrations of nitrates in the air range from 1-8 $\mu g/m^3$. Very low concentrations of nitrates have been reported in the South Pacific (0.1 - 0.4 $\mu g/m^3$), and very high concentrations are reported in the Netherlands, where the monthly average nitrate concentration in the air is in the range of 1-14 $\mu g/m^3$ [3].

1.2.2 Food

Canned vegetables and meat are considered the main sources of nitrates and nitrites in the human diet. Meat products contain 2.7-945 mg nitrogen per kilogram, dairy products can contain up to 0.2-6.4 mg nitrogen per kilogram and some vegetables and fruits can contain up to 2.5 g/kg vegetables. The concentration of nitrates in vegetables and fruits is affected by the use of fertilizers and growing conditions (temperature, light intensity, soil quality).

The vegetables that contain the highest concentrations of nitrates are radishes, beets, lettuce and spinach. They can contain concentrations of up to 2.5 g/kg of vegetables, especially if they have been grown in greenhouses [3].

1.2.3 Water

Nitrogen compounds in water come from a multitude of sources, most of which are associated with human activities.

In surface waters, the concentration of nitrates is generally low, ranging from 0-18 mg/l, but in the case of industrial, agricultural or waste discharges, their concentration may increase. A seasonal fluctuation in the concentration of nitrates in the surface water was also observed, which increased during the rainy seasons.

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 [3].

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 [4].

Intensive agriculture, the use of chemical fertilizers and the improper storage of waste from animal farms are the main factors that have led to an increase in the amount of nitrogen in groundwater over the last 20 years. Once polluted with nitrates, the aquifer remains contaminated for decades, even if the source of nitrate is reduced or eliminated.

In the case of intensive agriculture, high doses of fertilizers can lead to the storage of nitrogen by plants. Thus, vegetables such as spinach and carrots are likely to accumulate nitrogen in their composition [5].

In most individual wells around the world, the concentration of nitrates is above 50 mg/l [3].

1.3 Effects of nitrogen compounds on the human body

Exposure to nitrogen concentrations in the air is considered to be negligible, as these concentrations are very low.

If the concentration of nitrates in the water is below 10 mg/l, the main source of nitrate ingestion is food. If the concentration of nitrate in water intended for human consumption is above the limit of 50 mg/l, the main source of nitrates for humans is considered to be water [3].

In water supply systems, excess nitrogen compounds in raw water are undesirable because they can cause problems such as unpleasant odors and promote microbial development in the water distribution system.

Nitrogen or nitrate converted to nitrite in the body causes two chemical reactions that can cause adverse health effects: induction of methemoglobinemia, especially in children under one year of age, and the potentially carcinogenic formation of nitroamides and nitrosamines [3].

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 [2] [6] [7].

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 [7]

According to the EPA in the United States, the maximum allowable limit for nitrates in drinking water is 10 mg N/l [8].

The lethal dose of nitrates for humans ranges from 67 to 833 mg/kg body weight and 33 to 250 mg/kg body weight for nitrites [3].

1.4 Nitrogen sources in water

Water pollution with nitrogen compounds has its origins in a multitude of sources, most of which are closely related to human activity.

1.4.1 Surface waters

In the case of surface water, the main sources of pollution are:

- Surface leaks;
- Atmospheric dust.

In the case of surface runoff, nitrogen compounds can seep into water from the decomposition of plant or animal matter or fertilizers used in agriculture. Soil pollution with nitrates results from the fixation of atmospheric nitrogen by some plants so that nitrates are present in the soil even in the absence of nitrate fertilization. When nitrogen-based fertilizers are used in large quantities, larger than necessary for plants, the amount of nitrate in the soil increases, which can lead to pollution of water sources.

The presence of ammonium in water and the lack of nitrogen indicate recent pollution of water. When water contains both ammonium and nitrogen, pollution is assumed to have elapsed over a while. Lack of ammonium and the presence of nitrates and nitrites, a long-standing impurity.

In surface waters, a consequence of pollution by nitrogen-based compounds is eutrophication, which is defined as the excessive development of algae and higher plant species, caused by the enrichment of water with nutrients.

1.4.2 Groundwater

The main sources of nitrogen compounds in groundwater are:

- Improper storage of animal manure and household waste;
- Leaks from the sewer network;
- Septic tanks;
- Infiltrations.

Groundwater sources usually contain higher concentrations of nitrogen than surface water sources.

The main reasons that led to the increase in the concentration of nitrogen compounds in the groundwater are represented by the intensive agriculture and the lack of sewerage systems in the localities.

Intensive agriculture is considered to be the main source of nitrogen pollution in groundwater. As the demand for food increases and the principle of crop rotation is no longer sustainable, the soil is depleted of resources; This is why fertilizers are used to replenish plant nutrient reserves in the soil. Studies in many European countries have shown that most water sources contain high concentrations in areas where intensive agriculture is practiced. Concerning fertilizers used in agriculture, it is estimated that only 40-60% of the total amount applied is used by plants, the rest reaching water sources [9].

Nitrogen from underground sources has the property of accumulating, decontamination or depollution of groundwater sources being a very difficult process. Nitrates from natural groundwater come from the washing by rainwater of nitrates existing in the surface layer of the land, thus leading to pollution of the groundwater.

Nitrates are the most common contaminant of groundwater sources. It is estimated that in the United States, approximately 22% of groundwater sources are polluted with nitrates [5].

1.5 Pollution with nitrogen compounds in Romania

In Romania, the main characteristics of the state of the environment in agricultural areas have changed in the last 20 years. Intensive use of pesticides and fertilizers in some areas, improper irrigation and drainage practices, and incorrect application of mechanization work have affected environmental factors (especially soil and water). Eutrophication phenomena have occurred, especially after 1990, due to the use of chemicals in the past and due to poor management of waste generated by livestock farms as well as due to the lack of treatment plants. Most agro-zootechnical units (mainly small pig farms) do not have waste storage/treatment stations.

Problems with the quality of groundwater in rural areas are often caused by the storage of animal manure directly on the ground or in improper conditions, as well as the lack of sewage systems and sewage treatment plants. Periodically, animal manure mixed with household waste is stored in unauthorized places. As a result, large areas of agricultural land are occupied by waste that pollutes soils and waters. Under these conditions, the leachate seeps into groundwater or drains into surface water [10].

1.5.1 Nitrates Directive. The general framework and the situation in Romania

As a member state of the European Union, Romania is obliged to implement all EU directives, such as Directive 91/676 / EEC on the protection of waters against pollution caused by nitrate from agricultural sources, as amended by Regulation (EC) no. 1882/2003.

Legislative instruments for the protection and management of water resources have been promoted in the European Union.

Directive 91/676 / EEC on the protection of waters against pollution caused by nitrates from agricultural sources (Nitrates Directive) has as its general objective the reduction of water pollution. This is one of the first European environmental programs developed to improve water quality [10] [11].

The Directive aims to reduce pollution caused or induced by nitrogen from agricultural sources and thus to protect drinking water resources and aquatic ecosystems. The Nitrates Directive appeared in response to the recognition in 1980 that the use of manure as fertilizer in a fast-growing agricultural sector had negative effects on water quality and ecosystems, the application of manure leading to eutrophication of lakes and rivers. The directive was enacted in 1991 and its implementation began in the late 1990s.

The implementation of the Directive is achieved through the following actions, which must be carried out by all EU Member States:

- Water quality monitoring;
- Identification of polluted water bodies;
- Defining as groundwater all groundwater that reaches or tends to reach concentrations higher than 50 mg/l;
- Designation of vulnerable zones - all land surfaces that drain water to bodies of water affected by nitrogen pollution;
- Drafting and implementing codes of good practice and the Action Plan;
- Reviewing the extension of vulnerable areas to nitrates and analyzing the effectiveness of the implementation of the Action Program every 4 years;
- Conducting courses to implement the Code of Good Practice [10].

According to the Framework Directive 2000/60/EC, water is defined as a heritage that must be protected, treated and conserved as such.

The first classification of vulnerable and potentially vulnerable areas in Romania regarding nitrate pollution was made in 2003 by ICPA (Research Institute for Pedology and Agrochemistry) together with the National Administration "Romanian Waters". In this designation, the areas vulnerable to nitrates from agricultural sources represented 255 localities in Romania, which represents 8.64% of the country's surface.

In 2008, the areas vulnerable to nitrogen pollution were redetermined, considering the localities located in potentially vulnerable areas and areas vulnerable to nitrogen pollution in 2003 [13].

According to the European Commission, each Member State may designate areas vulnerable to nitrogen pollution to implement measures to reduce pollution or to apply such measures throughout the territory. In Romania, the second method was used.

The European Commission's 2010 report on the state of implementation of the Nitrogen Directive shows a decline in pollution in many countries. However, the report points out that in several regions, the level of nitrogen concentration in groundwater is worrying: Estonia, northern Italy, Slovakia, Spain, Romania, Malta and Cyprus. High concentrations have been identified in surface waters in Malta, Belarus, Belgium and France [14].

In Romania, the implementation of the Directive has not been very easy mainly due to non-compliance with the established programs.

The main factors that have negatively influenced the implementation of the directive are:

- Lack of financial resources;
- Failure to achieve a high-performance information network between monitoring and control factors;
- The presence of historical pollution [10].

The European Commission's 2015 report on the state of implementation of the Nitrates Directive shows an improvement in groundwater and surface water quality but stresses that further efforts are needed to address the issue of water pollution in EU Member States with nitrates.

The European Commission's Report on the Implementation of Directive 91/676 / EEC, based on Member States' reports, was issued in October 2021. According to this report, the following measures are needed:

- The Member States must identify polluted areas and designate them as nitrogen-vulnerable areas and implement mandatory measures in those areas or may choose to apply the actions throughout all their territory. Austria, Denmark, Finland, Germany, Ireland, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Romania, Slovenia and Belgium (limited to Flanders) chose the second approach;
- Most states have adopted new or revised action programs in the 2016-2019 reporting period. Updates are needed for Belgium, Cyprus, Finland and Romania;
- In some Member States there are areas where pollution is not sufficiently addressed: Bulgaria, Cyprus, Estonia, France, Italy, Portugal and Romania;

According to the report, the European Commission will develop an integrated action plan on nutrient management based on the Zero Reduction Action Plan in 2022 [15].

2 Technologies used to reduce the concentration of nitrates in drinking water

2.1 General elements

Over time, several methods have been developed to reduce the concentration of nitrates in water intended for human consumption.

If the water to be treated contains a concentration of nitrates above the limit required by law, several water treatment options can be considered, using processes such as reverse osmosis, ion exchange technologies, biological processes, or, the mixture of water with high nitrate content with better quality water (low nitrate content), resulting in water-containing nitrates in concentration below the limit imposed by the legislation in force [16].

If there is an alternative source of water that can provide the required amount of water, and the quality of the water from this source is higher than that from the source polluted with nitrates, the alternative source of water should be selected and temporarily or permanently abandon the source with a high concentration of nitrates.

In most cases, the use of an alternative source of water is not possible, and it is mandatory to treat the water before it is distributed to consumers. The main treatment processes applicable to sources that exceed nitrates are reverse osmosis, processes that use ion exchangers, electrodialysis, and biological processes.

2.2 Reverse osmosis

Reverse osmosis is a physical process that involves the passage of water through a semipermeable membrane that can retain almost the entire amount of salts in the water. The process takes place at pressures higher than the osmotic pressure and the process results in permeate (ultra-pure water) between 65% and 80% and concentrate (20% -35%), regardless of the quality of the raw water.

In general, reverse osmosis is applied only at a partial flow rate, so that the mixture of the permeate with the raw water that does not pass through the osmosis plant results in water that falls within the limits imposed by the legislation in force on nitrates and is not remineralization required. The fundamental problem is the formation of the concentrate, which must be treated or managed properly. The process has a very high degree of automation, which makes it suitable in rural areas, without the need for a permanent operator with a high degree of qualification [2] [17] [18].

2.3 Ion exchangers

Ion exchange is based on masses of ion exchangers specially dedicated to nitrogen retention. However, the process has several limitations due to the concentration of chlorides in the water, as the nitrogen ion in the water is exchanged with the chloride ion, grafted onto the ion exchange resin. In this way, situations may occur in which the concentrations of nitrates are reduced, but in the treated water there are excesses of chlorides. Also, although anionites have been designed to be selective, there is still a competitive action given by sulfate and bicarbonate ions. This is a disadvantage, especially in the case of groundwater treatment, which generally contains high concentrations of bicarbonates, which, when competing with the nitrogen ion, lead to premature depletion of the anionite's exchange capacity.

When the exchange capacity is exhausted, the anionite mass must be regenerated, requiring a significant consumption of brine for regeneration. The regeneration results in significant amounts of brine with a high nitrogen content, which in turn is a difficult waste to manage. The concentration of nitrates in the effluent is not constant over a cycle but increases progressively as the anionite exchange capacity decreases. The process requires permanently qualified operating personnel.

2.4 Electrodialysis

Electrodialysis is a process that is not widely used. It is suitable for treating low water flows. In the water treatment process, electrodes are inserted into the volume of water and a direct or alternating current is applied between the electrodes, leading to electrodialysis of the water [6] [16].

2.5 Denitrification of water using biological processes

Biological processes are based on the development of specific biomass for denitrification. In the process it is necessary to add a food source for bacteria, methanol is usually used. The use of an external carbon source in the process means an increase in the concentration of total organic carbon and implicitly the risk of THM formation, in the process of disinfection with chlorine, which is anticarcinogenic. Consequently, additional water treatment processes are needed to retain the introduced organic carbon as a food source as well as the resulting metabolites in the process. The process is unstable, so it must be controlled very rigorously and it is very sensitive to inhibitory substances. The process of initiating and restoring biomass takes a long time, and if the biomass is affected, the process must be stopped during its regeneration [19].

2.6 Biofilters

2.6.1 Development of the process

Biological filtration is a technology used to remove pollutants from a liquid using a bioreactor containing living matter to capture and degrade biological pollutants from the fluid. Frequent uses of biofilters are municipal wastewater treatment, industrial water treatment and biological oxidation of airborne contaminants.

Filtration of water for drinking purposes was first applied in Scotland around 1800, using slow sand filtration. Following the spread of cholera outbreaks in Europe in the second half of the century and the discovery that water filtration prevents some waterborne diseases, water filtration has spread rapidly in Europe and North America.

The concept of a biological filter used in wastewater treatment originated in England in the late 1890s. The biofilter was filled with water at the top, the wastewater was left there for a while, the filter was emptied, there was a rest period, and the cycle resumed. A complete cycle lasted 12 hours, 6 hours of operation and 6 hours of rest [20].

In the early 1900s, rapid filtration technology was discovered in the United States. The technology has gradually started to replace the old filtration technology due to the lower footprint and the higher quality of filtered water. By the 1930s, filtering technology using fast filters had surpassed the technology using slow filters in terms of the number of treatment plants using that technology [21].

In the 1950s, plastic began to be widely used as a backing material in biofilters used in water treatment. The use of plastic has led to a reduction in the area required for the construction of biofilters [22].

Biofiltration was first implemented for drinking water in Europe in the 1970s. The technology has been adopted to remove organic matter from the water. In the 1980s, this technology also reached America, but the implementation of the technology was difficult due to the reluctance of the population to use a technology that involves the intentional growth of bacteria in the water supply system. However, this reluctance did not last long. With the changing regulations for drinking water in terms of turbidity, water disinfection and disinfection by-products, the benefits of using biofiltration have been recognized.

The following table presents a brief history of the use of biological filtration for water treatment [21].

Table. 2.1 Biofilters use in water treatment [1].

Year	Event	City / Country
1804	The first slow filters used to treat water	Scotland
1852	The first regulations for water filtration following the outbreak of the cholera epidemic	London
1870	The first slow filter treatment plant in the USA	Poughkeepsie, NY
1880	The first fast filter treatment plant	Sommerville, NJ
'80	The cholera epidemic in Germany is leading to the development of water filtration technology	Germany
1913	Production and marketing of granular activated carbon in the United States begins	US
1925	600 fast sand filters are in operation compared to 50 slow sand filters in the US	US
1929	The first activated carbon filter	Hamm, Germany
1930	The first activated carbon filter installed in the public water supply system	Hopewell
1961	The first activated carbon filter in the USA	Bay City
'70	Biofilters are beginning to be used in Europe	Europe
1978	U.S. Epa proposes the use of activated carbon filters for drinking water	US
1980	Biodenitrification is beginning to be used in Europe	Europe
1982	"Biological activated carbon" is published, which describes the practices used in Europe and promotes the biofiltration technology in the USA	US

Year	Event	City / Country
1984	The work "Achieving biological stable water" by Rittmann and Snoeyink - AWWA is published	US
1988	The book "Biological processes in Drinking water treatment" - AWWA is published	US
1989	New regulations regarding drinking water that require water filtration as a mandatory process	US
2008	AWWA establishes a committee for the biological treatment of water	US
2013	The first symposium dedicated to the biological treatment of water for drinking	US

2.6.2 The Biofilm

The ability to form biofilm is a universal attribute of microorganisms. The development of the biofilm can take from a few days to a few months, this period is mainly influenced by the intake of organic substances. In both natural and artificial systems, biofilm consists of microbial cells transported in a fluid. When transported by solid, microbial cells can adsorb, grow and multiply using the solid medium as a support layer, thus forming Extracellular Polymeric Substances (EPS) [2] [23].

The structure of the EPS matrix of biofilms consists of DNA, extracellular polysaccharides (40-95%), and proteins (5-60%). The structure of the biofilm is constructed in such a way as to allow the access of nutrients throughout the biofilm [23] [24].

The most important processes that appear in the activity of a biofilm are:

- Fixation of microorganisms;
- Biomass development;
- Detaching the biofilm from the holder.

2.6.3 Substrate

Substrates can be made of natural materials such as gravel, sand, granular activated carbon, etc., or artificial materials, most often plastic substrates are used, as they offer advantages given by easier maintenance and lightweight.

The most important features for choosing media are:

- Specific surface - represents the surface available for the development of bacteria; the larger the specific surface area, the smaller the size of the biofilter;
- Three-dimensional distribution of the surface - it is indicated that water can flow through the biofilter in all directions, thus ensuring a uniform supply of nutrients;
- Clogging resistance - as much free volume as possible of the support medium increases its clogging resistance;
- Ease of cleaning of the environment - to prevent disruption of the process, it is necessary that the support environment can be cleaned easily and quickly;
- Stiffness;

- Weight - for easy handling and as cheap a biofilter structure as possible, low weight of the support medium is recommended.

The most commonly used filtration media are granular activated carbon, anthracite, and sand. Biofilters can be composed of dual media, granular activated carbon, or anthracite over sand, but most biofilters work successfully using a single filter layer [21].

2.6.4 Biofilters operation

Submersible biofilters are used in treatment plants using biological denitrification processes. These, depending on the raw water supply mode, can be:

- Biofilter with ascending flow;
- Downstream biofilters.

3 Pilot installation and experimental protocol

Experimental research was performed at the laboratory study level on a pilot biological denitrification plant.

The main objectives of the experimental study were:

- Determining the time required for the natural development of a biomass capable of reducing the concentration of nitrates from raw water;
- Testing the efficiency of biological denitrification for three types of filter media used: activated carbon, ceramic granules, and sand;
- Estimation of the active height of the filter layer in the biofilter, to reduce the amount of nitrates in the water;
- Studying the behavior of biomass at changes in raw water parameters, high amounts of nitrates, low amount of carbon in water, and low retention time;
- Estimation of a nitrogen reduction capacity for developed biomass.

3.1 Experimental installation

The installation used for the experiments consisted of:

- Raw water basin;
- Submersible pump;
- Raw water distributor with the role of distributing the flow evenly in the 3 columns;
- 3 filter columns filled with different filter materials, each filter layer having a height of 1.00 m:
  - Sand filter, Dn 100 mm;
  - Activated carbon filter, Dn 100 mm;
  - Ceramic granules filter, Dn 100 mm;
- Filtered water drainage pipes.

The diagram of the pilot plant as well as photographs during the experimental cycles are shown in the following figures.

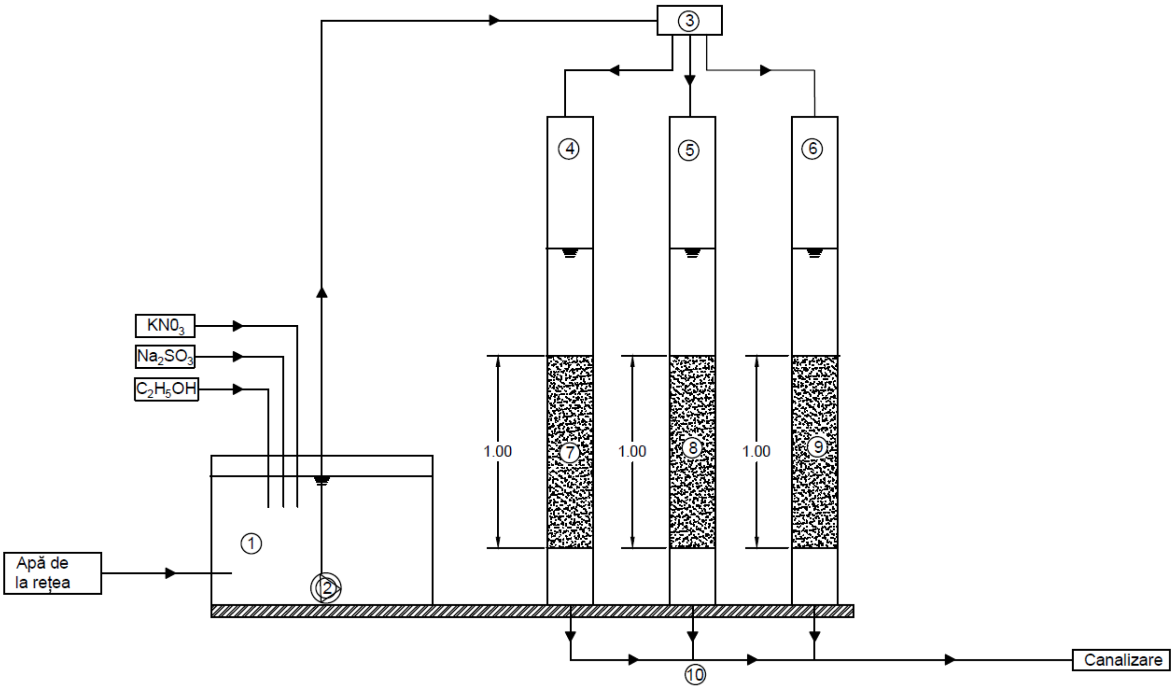

Figure 3.1. Experimental installation scheme.
1-Raw water tank, 2-Submersible pump, 3-Distributor, 4-Sand column, 5-Activated carbon column, 6-Ceramic granule column,
7-Filter layer-sand, 8-Filter layer-activated carbon, 9-Filter layer-ceramic granules, 10-Drained filtered water.

Figure 3.2. Photos of the experimental installation.

Left - Phase 1, Right - Phase 2.

The filter materials used in biofilters are activated carbon, sand and ceramic granules. The following table shows the characteristics of the filter materials used and the following figures show the filter materials used during the research.

Table 3.1. Filter media used.

Parameter	Activated carbon	Sand	Ceramic granules
Particle size (mm)	0.15-1.4	0.35-1.55	1.00-5.40
Filter layer volume (l)	7.86	7.86	7.86
Filter layer height (m)	1.00	1.00	1.00

Figure 3.3. Filter material - granular activated carbon granules.

Figure 3.4. Filter material - filter sand.

Figure 3.5. Filter material - ceramic granules.

3.2 Experimental stages

The experimental research was carried out in two stages:

- Stage 1 - Development of the denitrifying biomass;
- Stage 2 - Analysis of the efficiency of biofilters.

3.2.1 Stage 1 - Development of the denitrifying biomass

Its main objective was the development of stable denitrifying biomass, reducing the concentration of nitrate in raw water. The option of developing biomass naturally was chosen, without the addition of any biological preparation. The time required for biomass formation was 1 and a half months.

The water used to prepare the synthetic solution is represented by the water from the hot water network of the Colentina laboratory complex, the Department of Hydraulics, Construction and Environmental Protection, UTCB. This water was used because, after its analysis, a low concentration of chlorine was determined, a concentration well below the concentration of water in the cold water system and due to the higher temperature than that of cold water around 12-14°C. C.

In stage 1, for a rapid biomass development, a C: N: P ratio of 100: 5: 1 was ensured, a ratio used in sewage treatment plants [25]. Ethyl alcohol (C_2H_5OH) of agricultural origin and orthophosphoric acid (H_3PO_4) were used to achieve this ratio.

3.2.2 Stage 2 - Analysis of the efficiency of biofilters

The experimental determinations took place between 05.02.2021 - 02.03.2021. The objective of stage 2 of biofilters operation was to evaluate the efficiencies of reducing the concentration of nitrates in the water using different filtration media, but also to highlight the influence of the main factors on the efficiency of the process.

The raw water concentrations for which the tests were performed are 100, 150 and 300 mg/l. For concentrations of 100 and 300 mg/l, filtered water from 3 filtration cycles (3, 6 and 9 hours) was analyzed.

The raw water parameters considered for each experimental determination are presented in the following table.

Table 3.2. Raw water parameters during experimental cycles.

The experimental cycle	Nitrogen concentration (mg / l)	C: N ratio	Filtering speed (m / h)	Sampling frequency (hours)	Sampling frequency (hours)
Experimental cycle 1	150	13:1	2.54	2	3
Experimental cycle 2	100	13:1	2.54	3	3
Experimental cycle 3	300	13:1	2.54	3	3
Experimental cycle 4	150	1.3:1	2.54	-	-
Experimental cycle 5	150	13:1	5.08	-	-

The following experimental protocol was used for all experimental determinations:

- After the completion of phase 1 (for the first filtration cycle) or after the completion of an experimental cycle, a solution with a concentration approximately equal to the nitrate concentration used in the experimental cycle determinations was introduced into the plant.

- 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 experimental testing began.
- 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 for each experimental cycle were resumed.

3.3 Parameters analyzed

Water sampling was performed from each biofilter, both from the biofilter effluent and from its layers. For the samples taken, the following parameters were determined:

- Nitrate concentration using a DR 3900 spectrophotometer and Hach-Lange test kits;
- Oxidability (According to SR EN ISO 8467-2001);

The oxygen concentration in the raw water was also monitored so that it did not exceed 0.2 mg/l in the raw water.

4 The results of experimental research

The objective of stage 2 of biofilters operation was to evaluate the efficiencies of reducing the concentration of nitrates in the water using different filtration media but also to highlight the influence of the main factors on the efficiency of the process.

The raw water concentrations for which the tests were performed are 100, 150 and 300 mg/l. For concentrations of 100 and 300 mg/l, filtered water from 3 filtration cycles (3, 6 and 9 hours) was analyzed.

The raw water parameters considered for each experimental determination were:

- Cycle 1: nitrogen concentration: 150 mg / l, C: N ratio = 13: 1, filtration speed 2.55 m / h;
- Cycle 2: nitrogen concentration: 100 mg / l, ratio C: N = 13: 1, filtration speed 2.55 m / h;
- Cycle 3: nitrogen concentration: 300 mg / l, C: N ratio = 13: 1, filtration speed 2.55 m / h;
- Cycle 4: nitrogen concentration: 150 mg / l, C: N ratio = 1.3: 1, filtration speed 2.55 m / h;
- Cycle 5: nitrogen concentration: 150 mg / l, C ratio: N = 13: 1, filtration rate 5.10 m / h.

Sodium sulfite was used in the stoichiometric ratio of 10 mg of sulfite to 1 mg of oxygen to keep the concentration of dissolved oxygen in the water below 0.2 mg / l [26] [27] [28].

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

Table 4.1 Results obtained - reduction of nitrate concentrations.

Experimental cycle	Sub-cycle	Nitrates (mg/l)				Efficiency (%)		
		Raw water	Filtered water					
			Activated carbon filter	Ceramic granules filter	Sand filter	Activated carbon filter	Ceramic granules filter	Sand filter
1	**1**- 3 h	150	4.4	9.04	80.7	97.07%	93.97%	46.20%
	2 - 6 h		2.13	1.07	7.83	98.58%	99.29%	94.78%
2	**1** - 3 h	100	3.78	0.1	2.13	96.22%	99.90%	97.87%
	2 - 6 h		0.3	SLD*	SLD*	99.70%	99.99%	99.99%
	3 - 9 h		0.1	SLD*	SLD*	99.90%	99.99%	99.99%
3	**1** - 3 h	300	0.87	6.21	24.74	99.71%	97.93%	91.75%
	2 - 6 h		0.23	11.8	2.25	99.92%	96.07%	99.25%
	3 - 9 h		0.864	0.653	0.811	99.71%	99.78%	99.73%
4	**1** - 9 h	150	0.24	54.1	77.1	99.84%	63.93%	48.60%
5	**1** - 9 h	150	SLD*	0.387	SLD*	99.99%	99.74%	99.99%
Minimum efficiency (%)						**96.22%**	**63.93%**	**46.20%**
Medium efficiency (%)						**99.06%**	**95.06%**	**87.82%**
Maximum efficiency (%)						**99.99%**	**99.99%**	**99.99%**

* - The concentration of nitrates was below the limit of detection of the method used.

Thus, for all nitrate concentrations used, under a C: N: P ratio of 13: 1: 0.2, the biofilters reduced the concentration of nitrate in the water to values below the limits imposed by the legislation (50 mg / l) for most of the probes (23 out of 24).

In the case of reducing the carbon 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%.

Experimental cycle no. 5, which involved halving the retention time of biofilters, highlights the ability of biofilters to reduce the amount of nitrates even at a high filtration rate, with all 3 filters having very good results in reducing the amount of nitrates in the water.

Also, by doubling the filtration rate, a flow rate treated by each filter of 40 l/h was obtained (filtration speed vF = 5.10 m / h), thus resulting in an amount of nitrates in the influent of 6.0 g NO_3^- /h in each biofilter. Given that in the filtered water from each biofilter, the amount of nitrates is less than 0.5, and the retention time in the filter layers is 11.8 minutes, it results that the rate of reduction of nitrates in each filter layer is about 150 mg /l/h.

Following the analysis of the results, it can be seen that all 3 biofilters are effective in reducing the concentration of nitrates in the water. However, the activated carbon filter was the only filter that reduced the concentration of nitrates below the level required by law in all experimental cycles, including cycle no. 4, which involved reducing the amount of nutrients.

Sand biofilters and ceramic granules reduced the concentration of nitrates in filtered water with an efficiency of over 90% for all experimental cycles, except cycle no. 4, in which their efficiency decreased below 65% and the concentration of nitrates in the biofilter effluent was above the limit value imposed by the legislation.

This results in a lower sensitivity of the biomass attached to the activated carbon filter as opposed to the other two filter media analyzed, which have shown a higher sensitivity of the biomass attached to reduce the amount of nutrients.

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.

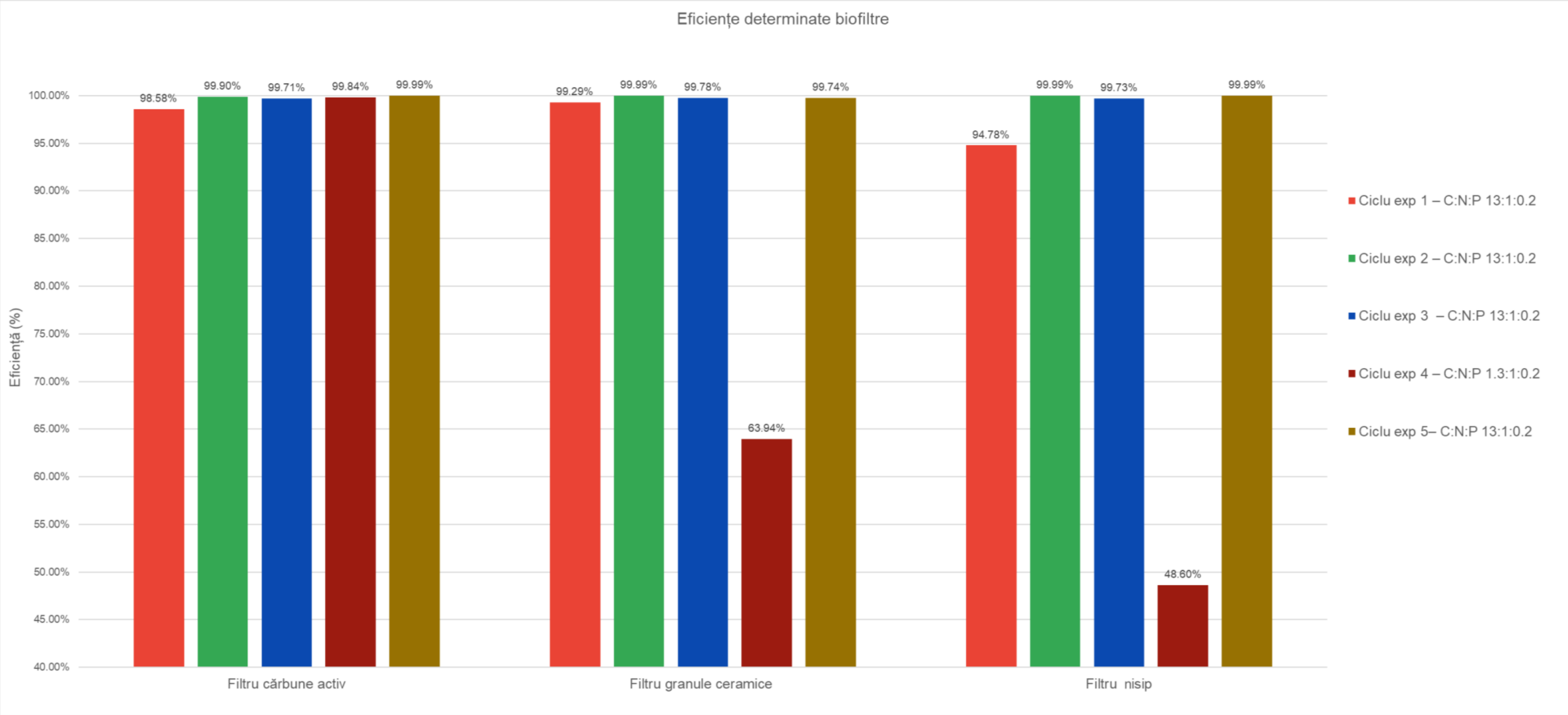

Figure 4.1. Nitrates reduction efficiency.

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.

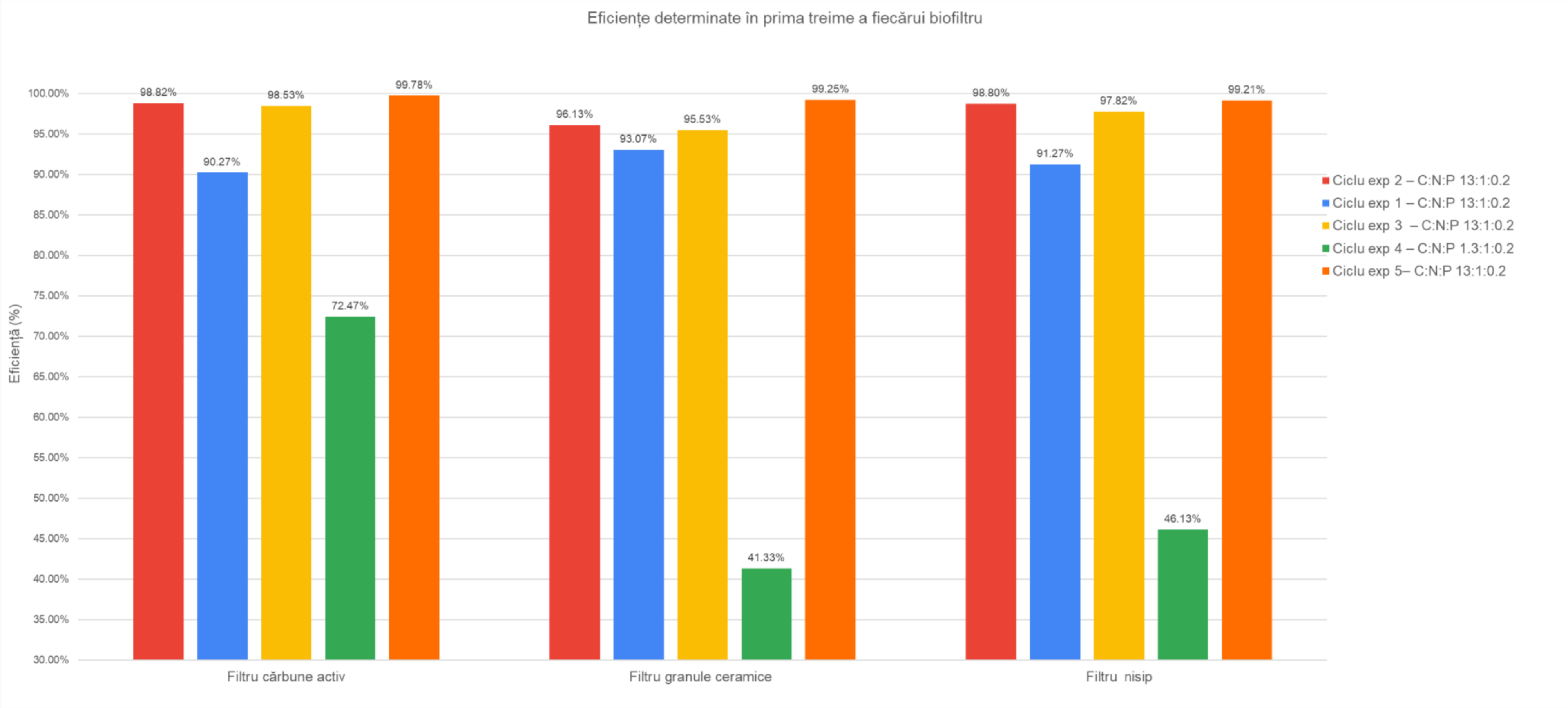

Figure 4.2. Nitrogen reduction efficiency in the first third of the layer.

The following table shows the results obtained in the experimental determinations for the oxidability of raw water and filtered water, respectively. The oxidizability of the water was determined by the water from the biofilter effluent.

Table 4.2. Results obtained - Oxidability of water.

Experimental cycle	Sub-cycle	Oxidability (mg $KMnO_4$/l)			
		Raw water	Filtered water		
			Activated carbon filter	Ceramic granules filter	Sand filter
1	1 - 3 h	56.88	63.2	75.84	72.68
	2 - 6 h		63.2	69.52	79
2	1 - 3 h	63.2	53.72	37.92	37.92
	2 - 6 h		63.2	69.52	69.52
	3 - 9 h		116.92	142.2	142.2
3	1 - 3 h	97.96	74.26	74.26	105.86
	2 - 6 h		47.4	53.72	126.4
	3 - 9 h		104.28	45.82	47.4
4	1 - 9 h	8.37	11.16	7.69	6.98
5	1 - 9 h	39.5	72.68	37.92	63.2

The amount of organic matter present in the filtered water was higher than in the raw water for most samples, which implies the need for a post-treatment step to eliminate it, as this increase can lead to oxidability values above the maximum concentration allowed by law. If the concentration of organic matter in the water is high, even if it is not above the maximum allowed by law, there is a risk of THM forming in the water during the disinfection process with chlorine, a potentially carcinogenic compound.

Increased oxidability of filtered water has been observed in other similar experiments. Thus, in 1998, Bruce O. Mansell and Edward D. Schroeder observed an average increase of 16 mg/l in the oxidability of filtered water compared to the value of oxidability in raw water [29]. In 2000, Greenan et. al. observed an increase in oxidizability of water in the range of 0-45 mg/l in the case of denitrification using heterotrophic bacteria [30].

Also, to result in quality effluent, treatment plants using biofiltration in Europe and the United States have included post-treatment steps to remove compounds resulting from filtration [31]. The technologies that can successfully remove organic matter from water are:

Oxidation - The process of chemical oxidation in water treatment plants has become increasingly used. Chlorine was originally used as an oxidizing agent but, due to the risk of forming products that are dangerous to human health, it has become less and less used. Ozone gives good results in oxidizing water. It can be used to remove organic matter, odors, taste, and watercolor [31].

Adsorption on activated carbon - The adsorption method uses the structure of materials with a large specific surface area that has the role of retaining organic substances. Activated carbon filtration is an efficient, energy-efficient process. It is used to remove color, odor and retain organic matter from water [32].

Membrane Filtration - Nanofiltration and reverse osmosis are processes that use membrane filtration and can be used to remove organic matter from water [33]. Although it provides very good results in the elimination of organic matter, the energy costs are very high. Also, the concentration resulting from filtration is difficult to manage.

5 General conclusions

A pilot biological denitrification plant was developed during the experimental research. It consisted of 3 biofilters, which contain different filtration media: activated carbon, ceramic granules and sand.

The objectives of the experimental research were:

- Determining the time required for the natural development of a biomass capable of reducing the concentration of nitrates in raw water;
- Determining the efficiency of biological denitrification for three types of filtration media used, activated carbon, ceramic granules and sand;
- Estimation of the active height of the filter layer in the biofilter, to reduce the concentration of nitrates in the water;
- Studying the behavior of biomass at changes in raw water parameters, high amounts of nitrates, low amount of carbon in water, and low retention time;
- Estimation of a nitrogen reduction capacity for developed biomass.

The analysis of the experimental results obtained during the experimental testing highlights the following conclusions:

- The time needed for biomass to develop in downflow biofilters with fixed biomass is around 6 weeks;
- For a C: N: P ratio of 13: 1: 0.2, the process is very effective in reducing the amount of nitrates regardless of the amount of nitrates influential, under controlled conditions, thus indicating high flexibility in operation, given that the sources record concentrations nitrate variables over time;
- The oxidability of the treated water is higher than the maximum permissible concentration required by the legislation in force for all water samples. It is thus necessary to provide post-treatment systems for the reduction of organic matter in water, after the biological reduction of nitrates in water;
- In the case of reducing the C: N ratio by 90%, sand and ceramic granule biofilters could not reduce the concentration of nitrates in the water below the legal limit, but the activated carbon biofilter had very high efficiency in reducing nitrates (99.84%) which indicating that the biomass attached to the activated carbon filter suffered much less from nutrient deprivation, most likely due to an accumulation of organic matter in the CAG micropores;
- Hydraulic retention time is one of the most important factors in sizing a biological reactor. In the experimental determinations, it was very short, 23.6 minutes, but the biofilters gave very good results in the case of halving this retention time, in the case of using a C: N ratio of 13: 1. As a result, high water flows can be treated using low-volume constructions. For example, using the conditions of the experimental research, at a retention time of 11.8 minutes, to treat a flow rate of 1 m^3/h results in a required volume of the filter layer of 0.2 m^3;
- Based on the results obtained, the highest amount of nitrates was reduced in the first 30 cm of the filter layer for each experimental cycle, so that the height of the active biological filtration layer in this test is about 30% of the height of total. This results in

a much lower required height of the filtration layer and thus a much lower investment cost than the usual technologies for water denitrification (reverse osmosis, ion exchangers).

Due to the very low hydraulic retention time, the low height of the biofilter active layer, the very high reduction capacity of the attached biomass and the high flexibility in reducing the amount of nitrates, regardless of the amount used in the experimental tests, can say that biological denitrification is an efficient solution that can treat water with high amounts of nitrates, take over large variations in loading and can be a cheaper and easier to implement solution than commonly used solutions such as ion exchanger technologies and reverse osmosis.

5.1 Elements of originality and personal contributions

The elements of originality and personal contributions developed in this doctoral thesis are:

- Comparative analysis of the efficiency of the three filter media in reducing nitrate concentrations by biological filtration processes:
  - Sand;
  - Ceramic granules;
  - Activated carbon;
- The fundamental parameters of the biological filtration process are established:
  - The time needed for the biological process to start – is 6 weeks;
  - Apparent filtering speed leading to maximum process efficiency: vF = 2.5 m / h;
- An optimal C: N: P ratio of 13: 1: 0.2 is established through experimental research, for which the process efficiency is very high and the effluent falls within the legal provisions regarding the concentration of nitrates, regardless of the filter medium, even at concentrations of 300 mg / l nitrogen in the influent;
- It is established the need to impose a post-treatment step to reduce the concentrations of organic substances that register high values following the biological process; such concentrations of organic compounds in biologically filtered water have also been reported by other researchers [29], [30];
- It was found that the biological filter with a granular activated carbon layer showed a high resistance to shock absorption in the nutrient supply, achieving close efficiencies of the nitrogen removal process, even at a 90% reduction in the amount of nutrient / organic substance added. The explanation of this phenomenon comes most likely from the fact that there are accumulations of organic substances in the micropores of activated carbon, which can be used in the situation of reduction or even interruption of nutrient supply;
- It has been established that the most active area in terms of biological process is represented by the first 30 cm of the filter layer.

The results of the doctoral thesis were capitalized by the publication of several articles, including:

- Matei, A., Vulpaşu, E., Racoviţeanu G. – "*Use of ion exchange technologies for nitrates removal from water intended for human consumption"*, Revista Română de Inginerie Civila, Bucureşti, 2022.

- Matei, Al., Racoviţeanu, G. – "*Review of the technologies for nitrates removal from water intended for human consumption*", 7th EENVIRO 2020 Conference - Sustainable Solutions for Energy and Environment, ID: 39, 2020.
- Matei, A., Racoviţeanu G., Vulpaşu, E.– "*Biological Denitrification of Water Intended for Human Consumption*", Modeling in Civil and Environmental Engineering, Bucharest, 2022.

5.2 Recommendations for future research

The following directions are considered to be a possible continuation of this paper:

- Analysis of the impact of temperature on nitrogen reduction efficiency;
- Analysis of the possibilities of reducing the oxidability of the biofilter effluent;
- Investigate and determine an optimal C: N: P ratio in terms of process costs to reduce the concentration of nitrates in the water.

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