

Start-up of biofilter for nitrification and effect of filtration rate on the ammonium removal efficiency in drinking water treatment on pilot plant

Dajana Dragić ^{a,*}, Dijana Drljača^a and Slobodanka Zorić^b

^a Department of Chemical Engineering and Technology, Faculty of Technology, University of Banja Luka, Stepe Stepanovića 73, Banja Luka 78000, Republic of Srpska, B&H

^b Water Production and Distribution Sector, 'Vodovod' AD, Sime i Ilije Partala 17, Banja Luka, Republic of Srpska, B&H

*Corresponding author. E-mail: dajana.dragic@tf.unibl.org

 DD, 0000-0003-4098-5679

ABSTRACT

Biological filtration is a process that can be used for ammonium removal from water. The removal of ammonium is based on the nitrification process. Nitrification depends on many factors, such as water quality and filtration process parameters. To investigate the influence of filtration rate on the nitrification efficiency, nitrifying biofilters were formed at the pilot plant in the industry for drinking water production from surface water. The new biological filters were started under optimal conditions for biofilm formation. After the biofilm formation, the influence of the filtration rate on nitrification was examined over 3 months (from November to January). The time required for the spontaneous growth of nitrifying bacteria on the new filters was around 70 days. The operating conditions at the start-up period were the continuous flow of water containing $\gamma(\text{NH}_4^+) \approx 1 \text{ mg/L}$, dissolved oxygen in a concentration $>9 \text{ mg/L}$, filtration rate $<1 \text{ m/h}$, inlet water temperature $>12 \text{ }^\circ\text{C}$ and pH from 7.8 to 8. After the complete formation of the nitrifying biofilm, the percentage of NH_4^+ removal was greater than 95% for all tested filtration rates, from 0.5 to 8.4 m/h, at $\gamma(\text{NH}_4^+)$ from 1 to 2.5 mg/L and raw water temperature from 13 to 7 °C.

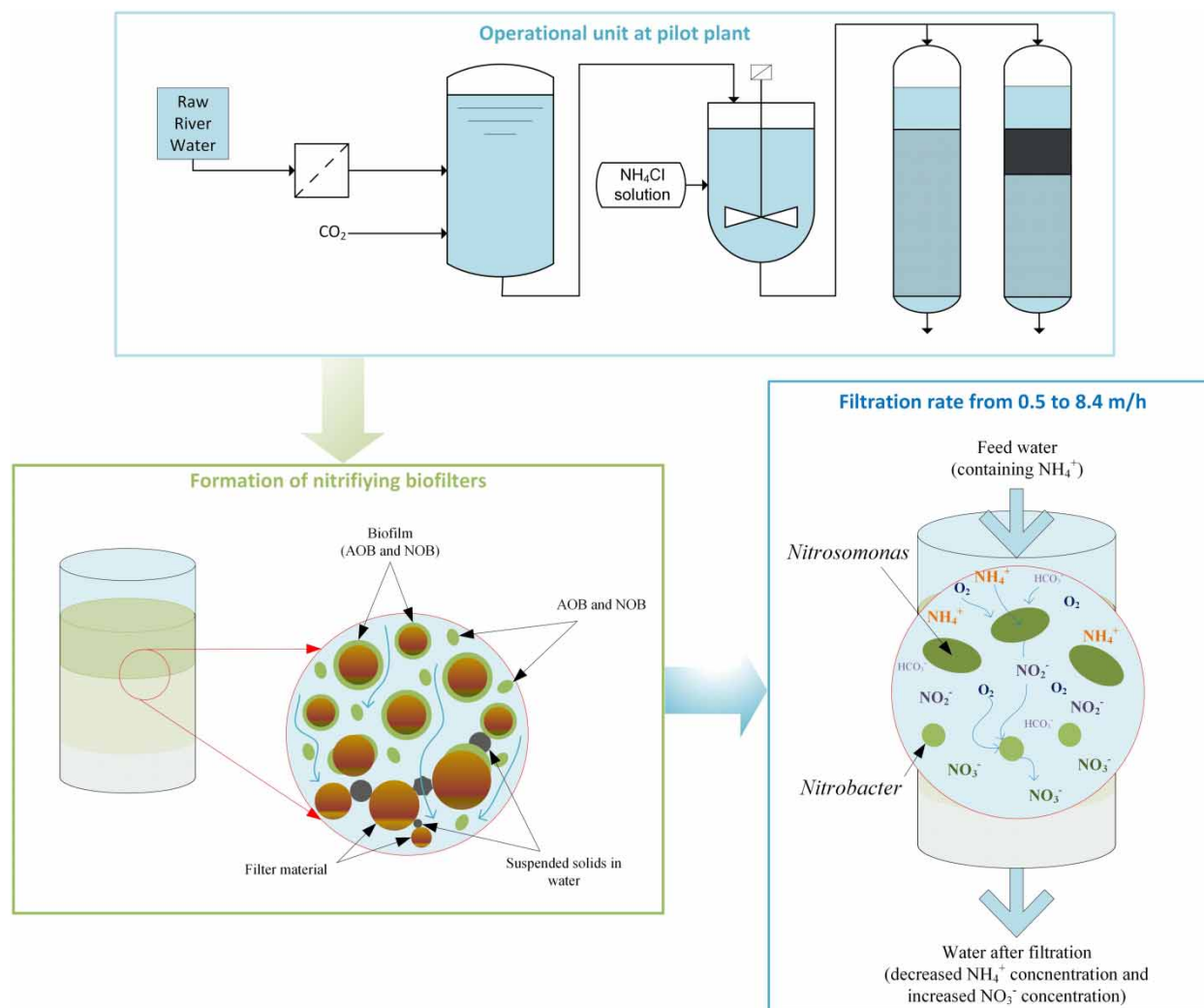
Key words: ammonium, drinking water, filtration rate, nitrification, pilot plant, temperature

HIGHLIGHTS

- Nitrifying biofilm was spontaneously formed on the surface of filter material at the pilot plant.
- Ammonium was removed by nitrification during filtration on rapid sand filters.
- Ammonium removal efficiency was higher than 95%.
- The complete nitrification was achieved even at the highest filtration rate of 8.4 m/h.
- Nitrification efficiency decreased at a high filtration rate when water temperature dropped below 7 °C.

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GRAPHICAL ABSTRACT



INTRODUCTION

Inorganic nitrogen compounds in surface water can be present in three forms: ammonium nitrogen (N-NH_4^+), nitrite nitrogen (N-NO_2^-), and nitrate nitrogen (N-NO_3^-) (Hasan *et al.* 2020).

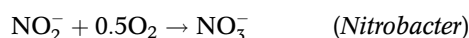
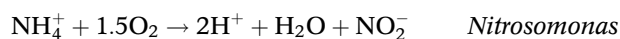
Ammonium is an inorganic nitrogen form found in surface water at $\text{pH} < 9$ (Radu & Racoviteanu 2021). The dominant forms of nitrogen in surface waters are mainly ammonium and nitrate, while the nitrite concentration is naturally low. The presence of nitrite is often the result of incomplete oxidation caused by a lack of dissolved oxygen in the water during the nitrogen oxidation cycle (Lee *et al.* 2014).

Ammonium can be naturally present in surface waters. Anthropogenic sources of ammonium are pollution caused by the discharge of industrial wastewater and sewage water, erosion of soil treated with nitrogen fertilisers, or decomposition of organic waste that contains nitrogen (Dey *et al.* 2022). The presence of ammonium in water affects the smell and taste of drinking water, reduces the effectiveness of chlorine disinfection, and can lead to the formation of pathogenic organisms during the water distribution (Maharjan *et al.* 2022). If present in drinking water, ammonium can lead to nitrification in water distribution systems, which can decrease dissolved oxygen concentration, decrease pH value, cause corrosion, or an accumulation of nitrites. Under aerobic conditions, NH_4^+ oxidises first to nitrite and then to nitrate. NO_2^- is much more harmful to human health because it causes methemoglobinemia (Maharjan *et al.* 2022). Maximum admissible concentrations (MACs) for nitrogen compounds are prescribed by different regulation guidelines. According to The Rulebook on health

safety of water intended for domestic consumption (Sl. Glasnik RS, 88/17) that is valid in the Republic of Srpska, MACs in drinking water are 0.5 mg NH₄⁺/L, 0.5 mg NO₂⁻/L and 50 mg NO₃⁻/L. For all the above-mentioned reasons, ammonium must be removed in drinking water treatment.

Ammonium can be removed from water using different chemical and physical (breakpoint chlorination, ion exchange, air stripping, membrane filtration) or biological treatments (Hasan *et al.* 2020; Dey *et al.* 2022; Maharjan *et al.* 2022). Each of these treatments has different advantages and disadvantages. One of the crucial factors determining the choice of applied technology in drinking water treatment is environmental and health safety and financial cost. Those treatments that do not require chemical reagents in high concentrations have an advantage when choosing an adequate technology. Biological treatments are becoming more applied in this field, and more research is being done on developing such technologies and finding the optimal operating conditions for achieving the highest possible efficiency. Biological treatments are most affected by raw water quality parameters and operating conditions, such as dissolved oxygen concentration in raw water, pH, phosphorus concentration, alkalinity, temperature, filtration rate or empty-bed contact time (EBCT) (Zhang *et al.* 2009; Lee *et al.* 2014). Water quality parameters have a significant influence on the metabolism and formation of the cell structure during the growth and development of nitrifying bacteria (Zhang *et al.* 2009; de Vet *et al.* 2012; Lytle *et al.* 2013). Water temperature affects the rate of enzyme-catalyzed reactions and the rate of substrate diffusion to the cell during biological oxidation (Zhang *et al.* 2009). Filtration rate and contact time are the limiting factors that determine the rate and efficiency of ammonium ion oxidation (Drljača *et al.* 2016; Liu 2017b). The main disadvantage of biological treatments is that some of these parameters are not so easily controlled. For example, the raw water temperature can be increased and adjusted to the optimal temperature for biological processes, but this requires additional energy consumption in the plant. The other problem is the high filtration rate that can provide enough water to supply the population with drinking water that meets all regulatory guidelines. However, a high filtration rate that provides such supply capacities can be limiting for nitrification activity. On the other side, some water quality parameters can be easily adjusted, e.g., alkalinity and phosphorus concentration just by adding a certain amount of suitable salt.

The biological treatment of ammonium removal is based on the biological activity of organisms that oxidise ammonium to nitrate. This process of biological oxidation is called nitrification. Nitrification, which takes place in two phases, can be presented with the following stoichiometric equations (Radu & Racoviteanu 2021):



The process of nitrification in drinking water treatment, organisms that carry out oxidation, all the factors that affect the growth of nitrifying bacteria and the progress of nitrification have become an important topic of research in water treatment technology.

The experimental part of this research included the start-up of new nitrifying biofilters at the pilot plant. One of the aims of this research was to determine the time needed for the complete formation of nitrifying biofilm on the surface of two different filter materials in the period when the raw water temperature was lower than the optimal temperature of 30–35 °C (Shourjeh *et al.* 2021) that favours the nitrifying biofilm growth. The biofilm was formed from raw river water. Water quality parameters were not additionally modified, except for the additional dosage of ammonia salt to increase input ammonia concentration. Raw water temperature in the period of nitrifying biofilm formation was below 13 °C. That temperature is way below the optimal temperatures for the start-up period of nitrifying biofilters that can be found in the previously published papers. In previous research, water temperature is usually maintained above 25 °C (Yapsakli *et al.* 2010; Suprihatin *et al.* 2017; Subari *et al.* 2018). During the operating period, the water temperature can be lower, which was the case in the nitrification process simulated with tap water with a temperature in the range from 17 to 23 °C (Cai *et al.* 2014) and groundwater with a temperature of 14.8 ± 2.2 °C during the operating period (Lytle *et al.* 2013). In this research, during the operating period, the water temperature was in the range of 12.6 to 4.6 °C. After the start-up phase, the experiments were continued on the rapid filter to examine the influence of the filtration rate on the ammonium removal efficiency. The influence of filtration rate, i.e., EBCT on biological ammonia removal was analysed over a wide range of filtration rates, 11 different EBCT values. The influence of filtration rate was studied in the period when raw water temperature decreased to lower values, without additional heating load and temperature control, in comparison to other studies where the nitrification process was studied at few

different *EBCT* and controlled and steady temperature conditions (Lytle *et al.* 2013; Suprihatin *et al.* 2017; Hasan *et al.*, 2019). The results of this research can be used to assess the efficiency of the biological process for ammonium removal from surface water on rapid sand filters and the possibility of implementing this process in drinking water treatment plants.

MATERIALS AND METHODS

Pilot plant

The experimental part of this research was carried out at the pilot plant in the drinking water production plant 'Vodovod' AD Banja Luka. The pilot plant at 'Vodovod' AD includes all the main technological units necessary for drinking water preparation from surface water (mechanical filtration, pH correction, coagulation and flocculation, precipitation, filtration, ozonation, and chlorine disinfection). However, not all the available technological units were used in the experiments performed in this research. The water preparation process was simplified. Water treatment at the pilot scale included primary (mechanical) filtration at the water intake (removal of large, floating impurities such as branches and leaves that can be found in surface water), pH correction and filtration on rapid sand filters. One additional unit was added before the filtration. That was the operation of feed water preparation in the mixer, where a solution of ammonium salt was added to the raw river water.

The pilot plant used for the experimental part and the operations performed during this research are shown in Figure 1.

Two different filtration columns were used in this research. The dimensions of the filtration columns are identical, but they were loaded with a different filtration material. The first filtration column (marked as F_1) was loaded with quartz sand, and the second (marked as F_2) was loaded with a combination of two different filtration materials, quartz sand and anthracite.

The filters used in these experiments are filters that match the specifications of the filters used in the main plant at 'Vodovod' AD Banja Luka which supplies the entire area of the city of Banja Luka with drinking water.

The specifications of the filters and filter material are given in Table 1.

During the experiments, filter backwashing was performed when the filtration rate decreased, and turbidity appeared in the effluent. Filter backwashing was performed when turbidity rose above 1 nephelometric turbidity unit (NTU), which is the limit value for turbidity in drinking water according to regulatory guidelines for drinking water (Sl. Glasnik RS, 88/17) or when the flow rate dropped below the set value due to accumulation of suspended solids in the filter. Backwashing was carried out by passing compressed air through filter material in an up-flow. Then, clean water was pushed through in an up-flow

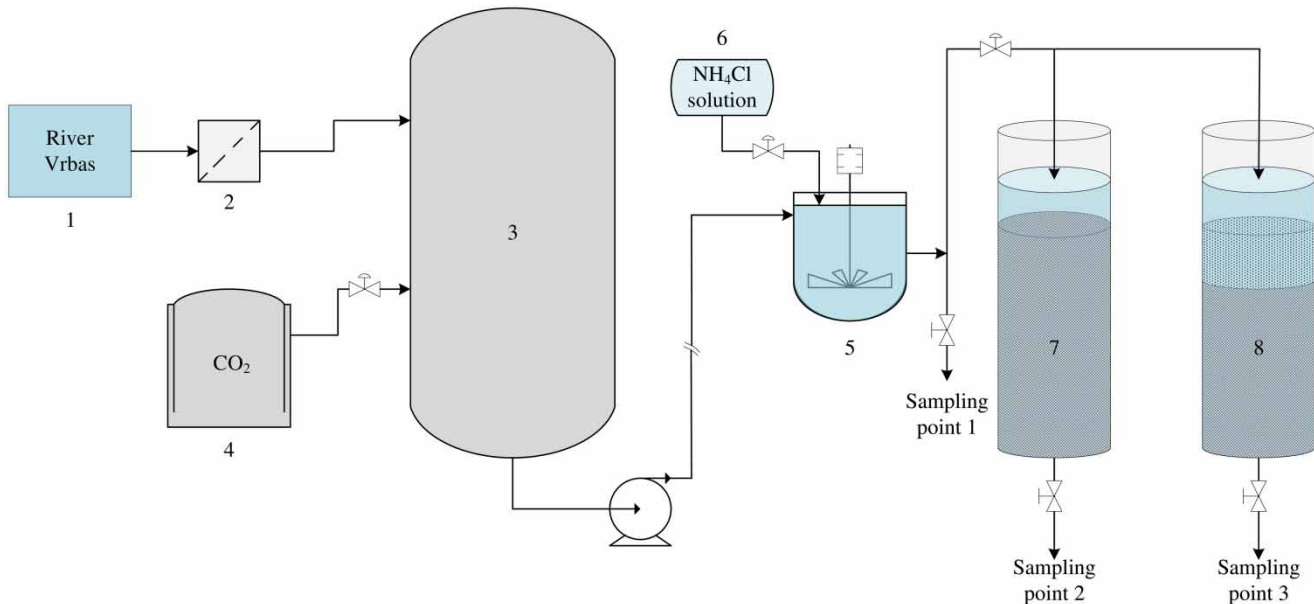


Figure 1 | Scheme of the pilot plant: 1. Water intake (river Vrbas); 2. Mechanical lattice filters; 3. pH correction with CO₂; 4. CO₂ pressure tank; 5. Mixer (preparation of feed water); 6. NH₄Cl solution tank; 7. Filter F_1 (quartz sand); 8. Filter F_2 (quartz sand + anthracite).

Table 1 | Specifications of filtration columns at the pilot plant

Specification	F ₁	F ₂
The total filter bed height (m)	1.20	1.20
Filter diameter (m)	0.39	0.39
Filtration media, height (m)	Quartz sand ($h_{\text{total}} = 1.20$ m)	Anthracite ($h_2 = 0.40$ m) Quartz sand ($h_1 = 0.80$ m)
Size of filtration medium particles (mm)	0.7–1.2	1.6–2.5 (anthracite) 0.7–1.2 (quartz sand)
Height of water level above filter material (m)	1.5	1.5

to wash the accumulated impurities (suspended particles) from the filter. The water from washing and suspended particles and impurities were drained over the overflow on the filter columns through the drainage channel into the sewage system.

Feed water

Feed water was prepared with the addition of NH_4^+ in raw water from river Vrbas because this river water did not naturally contain ammonium. Ammonium was continuously dosed in the form of NH_4Cl solution into the raw water in the mixer (Figure 1). After mixing, water with NH_4^+ was injected into the filtration columns. The concentration of NH_4^+ was varied during the performed experiments. However, the maximum values of the inlet concentration were limited and defined by the presence of dissolved oxygen in the raw water of Vrbas, which is necessary for the complete NH_4^+ oxidation. Additional water aeration was not performed during the pilot plant experiment.

The pH value and temperature of the water are critical factors which influence the nitrification process efficiency. Therefore, these water parameters were also measured and monitored daily.

Correction of the pH of the raw water of the Vrbas River was done with the injection of CO_2 into the water tank. This operation was performed after the water intake. The pH values of surface water before CO_2 addition were around 8.2, and after pH correction, values ranged from 7.8 to 8, which is the optimal value for the nitrification process (Shourjeh *et al.* 2021).

The water temperature was varying, and the change depended on the climatic conditions and weather that prevailed during the period in which the experiment was performed. Adjusting the temperature of the raw water by adding heat to the system was not performed in this work. However, the temperature change, a critical factor affecting nitrification efficiency, was not ignored during the experiment. Raw water temperature measurements were taken daily, and its influence on nitrification was monitored.

The water used for filter backwashing was produced at the pilot plant. After passing through the filters, that water was stored in tanks and used for backwashing. Disinfection was not carried out in that water, so the residual chlorine from the water would not prevent the biofilm growth, inhibit or stop the activity of nitrifying bacteria that had formed up to that time.

Start-up of nitrifying biofilters

Nitrifying bacteria (ammonium and nitrite-oxidising bacteria) were formed at the pilot plant by the constant flow of raw water from the river Vrbas, to which ammonium was added continuously. From the beginning of the experiments, ammonium was constantly maintained in the feed water, with occasional changes in the inlet concentrations. Feed water prepared this way was continuously passed through filters in the start-up period, from August through the end of October. The first goal was to naturally form a biofilm of ammonium and nitrite-oxidising bacteria that will conduct nitrification on a solid substrate, i.e., filter material.

The flow rate in this phase was maintained at low values, around 60 L/h, to enable the growth and fixation of nitrifying bacteria to the solid surface and to prevent the biofilm from being washed away under the influence of higher filtration rates, greater than 1 m/h, or even higher filtration rates specific for rapid sand filtration (Hasan *et al.* 2020). A lower flow rate in the start-up phase also reduced significant raw water losses.

During the experiment, the temperature and pH of water were monitored, the input concentration of ammonium was controlled, and the flow rate, i.e., the filtration rate. The water was not aerated since it is surface river water that contains sufficient concentrations of dissolved oxygen for the oxidation of NH_4^+ that was added to the feed water.

After the establishment of the nitrification process, based on the changes in concentrations of N compounds in the effluent regarding concentrations in influent, the consumption of oxygen for the oxidation of NH_4^+ at different inlet concentrations of NH_4^+ was also monitored. Dissolved oxygen was determined in the inlet water and water after filtration at the outlet of filters F_1 and F_2 .

Table 2 shows the main parameters that represent feed water quality parameters and filtration process parameters that affect the efficiency of nitrification, which were maintained during the biofilter start-up period.

Alkalinity is an important factor affecting nitrification because hydrogen carbonate ions are a source of inorganic carbon that is necessary for the growth and development of the cell structure of nitrifying biomass. Also, the buffering capacity of water, which originates from alkalinity is very important and it is used to neutralise hydrogen ions that are produced in the reactions of biological oxidation (Zhang *et al.* 2009). Phosphorus, as one of the essential micronutrients, also has an important role in the life cycle of nitrifying microorganisms (de Vet *et al.* 2012). The negative impact of phosphorus deficiency is noticeable when the water temperature drops, and then the phosphorus deficiency becomes a limiting factor for nitrification.

The influence of filtration rate on the nitrification process

The influence of the filtration rate on the efficiency of the nitrification was monitored on the dual media filter F_2 . Examined filtration rates ranged from 0.5 m/h ($EBCT = 2.4$ h) to 8.4 m/h ($EBCT = 0.14$ h), equivalent to the flow rate from 60 to 1,000 L/h. The flow rate of 1,000 L/h was the maximum flow that could be achieved at the pilot plant.

The filtration rate on filter F_2 was increased by ≈ 1 m/h and kept constant for 3–5 days to perform a certain number of repeated measurements for the examined water quality parameters. The temperature of the raw water gradually decreased during the experiment, from the initial 12 to 5 °C. The drop in temperature depended on the climatic conditions that affected the decrease in water temperature because the filtration rate experiment was performed in the autumn/winter period (from November to mid-January).

The main quality parameters of the feed water and the process parameters of filtration that were maintained during the experiment at the pilot plant are shown in Table 3.

Analytical methods and physical measurement

Standard analytical methods were used to monitor nitrification and ammonium removal efficiency. The nitrification was monitored and validated through measurements of the water quality parameters determined in the feed water and water after filtration. The sampling sites given on the pilot plant scheme (Figure 1) are *sampling site 1* – inlet, feed water; *sampling site 2* – water after filtration, outlet from F_1 ; *sampling site 3* – water after filtration, outlet from F_2 . All samples were analysed immediately after sampling.

Ammonium, nitrite and nitrate concentrations in water samples were determined by standard spectrophotometric methods on a UV/VIS spectrophotometer (Perkin Elmer UV/VIS Lambda 25). Used standard methods for the determination of nitrogen content were method with Nessler's reagent (without distillation) for the determination of N- NH_4^+ (ASTM D1426-08 2008), standard test method with sulfanilic acid for the determination of N- NO_2^- (Baird *et al.* 2017), and the standard

Table 2 | Filtration process conditions during the start-up of the nitrifying biofilter

Feed water specifications		Operating conditions	
NH_4^+ concentration (mg/L)	1–4.5	Filtration rate (m/h)	0.50
Alkalinity (mg CaCO_3/L)	≈ 200	EBCT (h)	2.4
Dissolved O_2 (mg/L)	≈ 11	Flow rate (L/h)	60
pH	7.8–8	Raw water temperature (°C)	12
Phosphorus ($\mu\text{g}/\text{L}$)	≈ 20		

Table 3 | Filtration process conditions during the filtration rate influence experiment

Feed water specifications		Operating conditions	
NH ₄ ⁺ concentration (mg/L)	1–4.5	Filtration rate (m/h)	1–8.4
Alkalinity (mg CaCO ₃ /L)	≈200	EBCT (h)	1.2–0.14
Dissolved O ₂ ^a (mg/L)	11–12	Flow rate (L/h)	60–1,000
pH	7.8–8	Raw water temperature (°C)	12–5
Phosphorus (µg/L)	≈10–20		

^aThe concentration of dissolved O₂ in water changes with temperature since the solubility of gases in water increases with decreasing temperature.

UV spectrophotometric test method for the determination of N–NO₃⁻ (Baird *et al.* 2017). The concentrations detected on the spectrophotometer were in mg N/L. Detected values of nitrogen were later converted to mg/L of NH₄⁺, NO₂⁻ and NO₃⁻ to be comparable with the MAC of these compounds in drinking water prescribed by the Regulations (Sl. Glasnik RS, 88/17).

The concentration of dissolved oxygen was determined by the standard Winkler method, and the alkalinity of the water was determined by the standard titrimetric method (Baird *et al.* 2017). Phosphorus concentration in raw water was determined by the standard method of spectrophotometric determination with ammonium molybdate and tin strip on a UV/VIS spectrophotometer (Shimadzu UV-1700) (Baird *et al.* 2017).

pH was determined with electrochemical measurements, together with the temperature, using the multifunctional measuring device *WTW MultiLineP4*. The temperature of raw water was also measured by the thermometric method using a thermometer in the temperature range from –10 to 50 °C.

The efficiency of the nitrification was expressed as a percentage of ammonium removed after filtration and is calculated according to the following equation:

$$\text{Removal (\%)} = \frac{\gamma_{\text{in}}(\text{NH}_4^+) - \gamma_{\text{out}}(\text{NH}_4^+)}{\gamma_{\text{in}}(\text{NH}_4^+)} \times 100\% \quad (1)$$

where removal (%) is the percentage of NH₄⁺ removed, γ_{in} is the mass concentration of NH₄⁺ in feed water (mg/L), γ_{out} is the mass concentration of NH₄⁺ in effluent (mg/L).

The flow rate through the filtration columns was measured using a rotameter. Based on the measured data of flow rate, the filtration rate (2) and EBCT (3) were calculated according to the following equations (Albers *et al.* 2018; Hasan *et al.* 2019):

$$v = \frac{Q}{A} \quad (2)$$

$$\text{EBCT} = \frac{V}{Q} \quad (3)$$

where v is the filtration rate (m/h); Q is the flow rate (m³/h); A is a cross-sectional area of filter column (m²); *EBCT* is the empty-bed contact time (h); and V is the total volume of the filter media bed (m³).

RESULTS AND DISCUSSION

Start-up of biofilters and growth of nitrifying biofilm

The first analysis of water samples after filtration was carried out on the fourth day after passing the water enriched with NH₄⁺ through the filters. In the beginning, only the change in the ammonium concentration was monitored, assuming that in the initial period, only ammonium oxidising bacteria (AOB) that oxidise NH₄⁺ to NO₂⁻ were formed. The growth of nitrite-oxidising bacteria (NOB) begins after the completion of the first phase in which nitrite is generated. As a result of the complete first phase of nitrification, an accumulation of nitrite occurs, which is necessary for the growth of NOB. Only after the growth and complete formation of NOB, complete nitrification is established, which is observed as a decrease in the concentration of ammonium and an increase in nitrate concentration (Teklerlekopoulou *et al.* 2013). Changes in the concentrations of NH₄⁺ during the start-up period are shown in Figure 2.

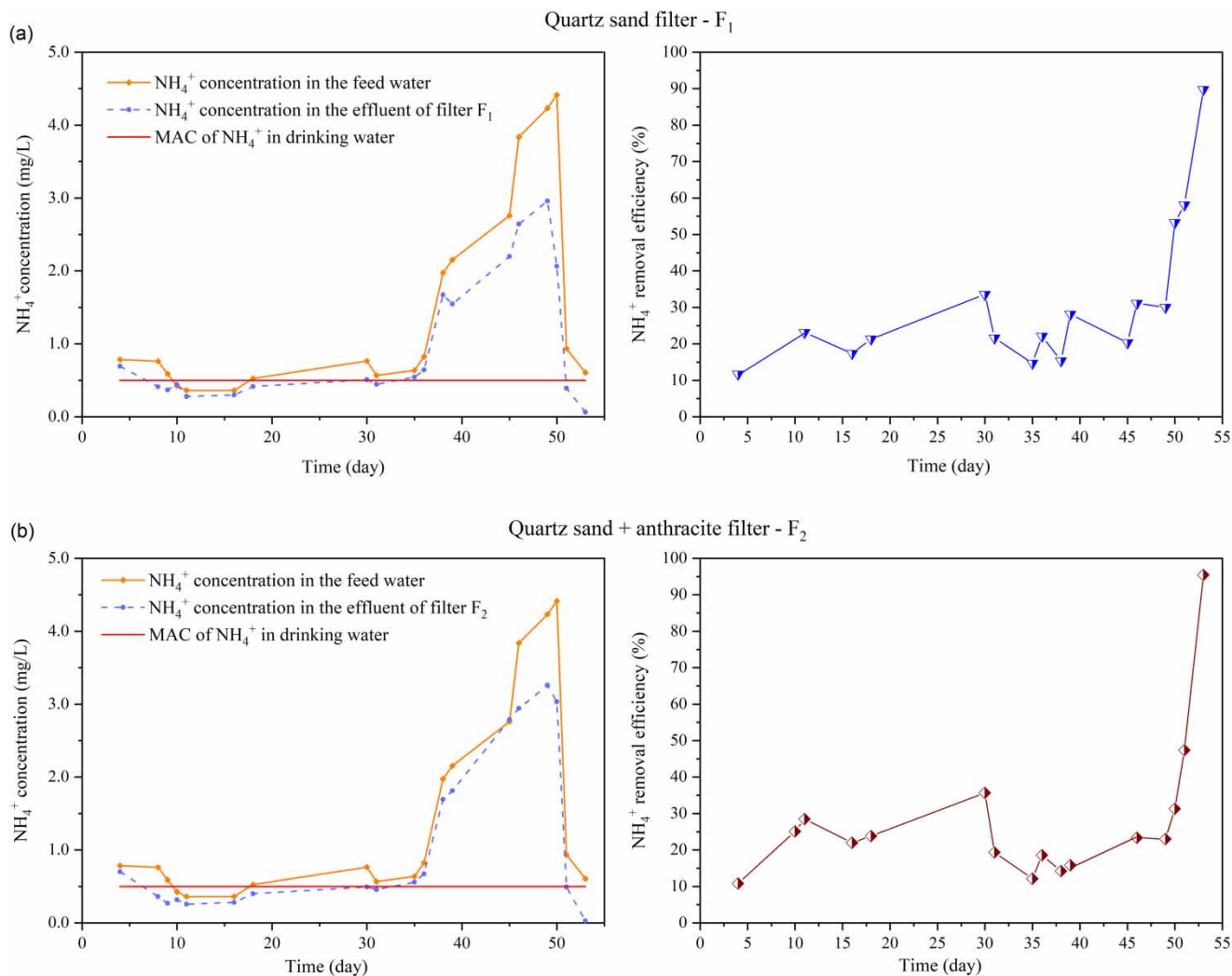


Figure 2 | Start-up of biofilters: (a) Change in NH_4^+ concentration in water samples and NH_4^+ removal efficiency on quartz sand filter F_1 ; (b) Change in NH_4^+ concentration in water samples and NH_4^+ removal efficiency on quartz sand + anthracite filter F_2 .

The filter inoculation period with AOB lasted 53 days (Figure 2(a)) under the following operating conditions: temperature around 12 °C, filtration rate of 0.5 m/h ($EBCT = 2.5$ h), NH_4^+ concentration in the range from 0.36 to 4.41 mg/L. Although the original goal was to keep the inlet NH_4^+ concentration constant at approximately 1 mg/L, variations in the inlet NH_4^+ concentrations were caused by irregular operation caused by malfunction of the pump used for the dosage of the ammonium chloride solution. However, in this phase, the constant presence of NH_4^+ was necessary for biofilm growth, so the dosage of NH_4^+ was not stopped to repair the pump. Higher input concentrations of NH_4^+ compared to the stopping the dosage due to reparations was a better alternative, because higher concentrations of NH_4^+ could accelerate biofilm growth (Cai *et al.* 2014).

Process parameters (filtration rate, raw water temperature, NH_4^+ concentration in feed water, pH, dissolved oxygen concentration) were identical for both filters. The water flow rate is an important parameter that significantly affects the activity of nitrifying bacteria and their growth, especially in the initial phase when a new biofilm is formed on the surface of the filtration material (Drljača *et al.* 2016; Liu 2017b). Therefore, the flow rate at the start-up period was maintained at 60 L/h.

A more significant decrease in NH_4^+ concentration in the water after filtration occurred on the 39th day after the start of the experiment. These results in the initial phase of biofilter start-up indicate that the nitrification has not yet fully started and that the biofilters are only in the initial phase of formation. The number of nitrifying bacteria present for efficient nitrification and high oxidation rate of NH_4^+ was insufficient, and their distribution density on the filtration medium was still very low at this period, based on the small changes in outlet concentration of NH_4^+ . Although AOB may be naturally present in raw surface

water and river systems, a certain period and optimal conditions are necessary for the growth and fixation of the bacteria for complete nitrification (Lee *et al.* 2014).

On filter F_1 (quartz sand), after 45 days, more visible variations in the output concentrations of NH_4^+ are registered. It was concluded that during that period, there was a change in the density of AOB that were distributed on the surface layer of the filtration material, which directly affected the increase in the oxidation rate of NH_4^+ (Lee *et al.* 2014; Tatari *et al.* 2016). Already on the 53rd day, the drop in the concentration of NH_4^+ is more noticeable because the outlet concentration is significantly lower than the inlet concentration.

A decrease in the concentration of NH_4^+ at the outlet of filter F_2 (quartz sand + anthracite) occurs on the 45th day after the filter is put into operation. In the following period, this concentration constantly decreases and becomes negligible in relation to the input concentration of NH_4^+ .

As shown in Figure 2, during the period of AOB formation, the input concentration of ammonia increased above 1 mg/L. Up to day 49, the percentage of ammonia removal was around 30% on F_1 . On day 50, at higher input concentrations, the ammonia removal was 53.3%. After that period, the ammonia removal percentage continued to increase. This may result from the assimilation of ammonia nitrogen into the cells of *Nitrosomonas* bacteria, whose growth occurs in this phase so that the loss of a certain amount of ammonia nitrogen is not only a consequence of oxidation. This conclusion agrees with the results of many authors who state that the loss of total nitrogen is more significant due to the assimilation of nitrogen in the cells of microorganisms if the input concentration of ammonium increases (Yu *et al.* 2007; Cai *et al.* 2014).

The decrease in the ammonium concentration in the effluent verified the beginning of the complete activity of AOB. The efficiency of the process during the experiment was expressed through the percentage of removed ammonium. The efficiency of NH_4^+ removal during filtration in the start-up phase of the biofilter is also presented in Figure 2.

The efficiency of NH_4^+ removal on the filters for the first 40 days was very low (the removal percentage at the first measurement was 11.7% on F_1 and 10.8% on F_2). From the 45th to the 53rd day, there was a significant increase in the removal efficiency on the quartz sand filter, so the removal efficiency on the 49th day was 77.3%. On the 53rd day (at the end of the phase of inoculation with AOB), the NH_4^+ removal efficiency reaches 89.8%. The removal efficiency on the combined, two-media filter increased more slowly than the sand filter, but at the end of the AOB growth phase, it had a higher value than the sand filter and was equal to 95.5%. Similar results in the efficiency of ammonium removal for the same filter materials were obtained by Drljača *et al.* (2016), who stated that nitrification was initially more efficient on quartz sand filter with a removal percentage of 80%, in comparison with 70% on the two-media filter. However, they stated that very quickly, there is an equalisation in the operation of the filters, with the removal percentage of ammonium over 95% on both filters.

When observing biofilm growth on F_1 and F_2 filter beds, these are different filtration surfaces on which a nitrifying biofilm was formed. Biofilm is mainly formed in the surface layers of the filter media. Lee *et al.* (2014) investigated the efficiency of NH_4^+ removal by the depth of the filter media. They found that the highest removal efficiency was achieved in the upper layers at a depth of up to 0.2 m. Bacteria in the deeper layers of the filter media do not have enough nutrients or dissolved oxygen for growth and activity (Lee *et al.* 2014; Liu 2017a; Subari *et al.* 2018). Therefore, we can conclude that the active biofilm was formed on quartz sand (filter F_1) and anthracite (filter F_2) because the upper layer of F_2 consists of anthracite with a height of 0.4 m. The particle size of quartz sand on the F_1 is 0.7 to 1.2 mm, compared to an anthracite particle size of 1.6–2.5 mm. The difference in the particle size of the filter materials resulted in the difference in the AOB formation rate. These minor differences in the start-up time and the oxidation rate of NH_4^+ are probably a consequence of the differences in the specific surface area of the different filtration media and their surface structure, which can primarily affect the number of available sites for the attachment of bacteria during filtration (Lájer 2012).

Growth of NOB and incomplete nitrification phase

The NH_4^+ removal efficiency increased after 50 days since the filtration of water enriched with NH_4^+ began. Complete oxidation of NH_4^+ caused nitrite accumulation in the effluent because NOB had not yet been formed. The accumulation of NO_2^- is a consequence of the incomplete process of nitrification, which is reflected in the incompleteness of the second phase, the oxidation of NO_2^- to NO_3^- . NOB (*Nitrobacter*) is formed after the complete formation of AOB. The growth of NOB can only occur once AOB increases the nitrite concentration in the water to a value sufficient for NOB growth and development. As nitrite was not initially present in the raw water in such high concentration needed for NOB development because the water of the Vrbas River does not contain naturally occurring nitrite, a certain period had to pass for a colony of

Nitrobacter to form from the nitrite generated from the first oxidation phase. After a significant drop in the concentration of NH_4^+ in the water after filtration was recorded, the presence of nitrate was also analysed in the samples. The beginning of the complete nitrification phase was monitored by measuring the change in the concentration of NH_4^+ and NO_2^- in the water at the outlet of the filters.

Figure 3 shows the results of measurements of NH_4^+ and NO_2^- concentrations during incomplete nitrification on filter F_1 .

Figure 3 shows that after the 50th day, faster oxidation of ammonium occurs on filter F_1 , and the drop in NH_4^+ concentration is visible in the water after filtration. Ammonium removal efficiency in the initial period still varied due to the growth, distribution of bacteria and their adaptation. After the adaptation of AOB in this phase, the percentage of ammonium removal ranged from 97.5 to 98.9% from the 65th to the 72nd day after the start of the filter, regardless of changes in the input ammonium concentration. This decrease in the concentration of NH_4^+ , with the simultaneous increase in the concentration of NO_2^- in the effluent, proves the activity of AOB and the complete development of the first stage of nitrification (Cai *et al.* 2014).

Figure 3 also shows that, in this period, there is an increase in the concentration of nitrite in the effluent from filter F_1 compared to the concentration of nitrite in the feed water. The nitrite oxidation rate is low, i.e., the second stage of nitrification takes place more slowly due to the lower number of NOB which were still in the growth phase. An increase in the input concentration of NH_4^+ in the feed water on the 67th day resulted in NH_4^+ removal efficiency greater than 97% but caused an increase in the NO_2^- concentration in the effluent. This also proved the completeness of the first phase and the activity of AOB. But, on the other hand, the lack of NOB for sufficiently fast oxidation of NO_2^- to NO_3^- . This phase during the biofilter start-up period defines the incomplete nitrification phase, which lasted 20 days for the quartz sand filter.

The two-media filter F_2 behaved in an almost identical way, which can be seen in Figure 4. The difference is that the period of incomplete nitrification on the two-media filter lasted 15 days, which is 5 days shorter than the quartz sand filter.

In the incomplete nitrification phase, water should still not be distributed to consumers due to higher concentrations of NO_2^- . The concentration of nitrite in the effluent from the quartz sand filter, for the days when measurements were done, did not exceed the MAC in drinking water (0.5 mg NO_2^-/L), which is prescribed by The Rulebook on health safety of water intended for domestic consumption (Sl. Glasnik RS, 88/17). On the two-media filter, during one measurement, there was one detected concentration of NO_2^- (0.54 mg/L) that exceeded the MAC for NO_2^- . As this was the start-up period of the biofilter, it was considered that the period of complete nitrification had yet to be established. Thus, water should not be distributed to consumers, not until the nitrite concentration had dropped to values less than or equal to those present in the feed water.

Incomplete nitrification, i.e., a delay in the completion of the nitrification process, is a phenomenon that occurs in any biological water treatment system (Albers *et al.* 2018). After the rise in nitrite oxidation rate and the establishment of a complete

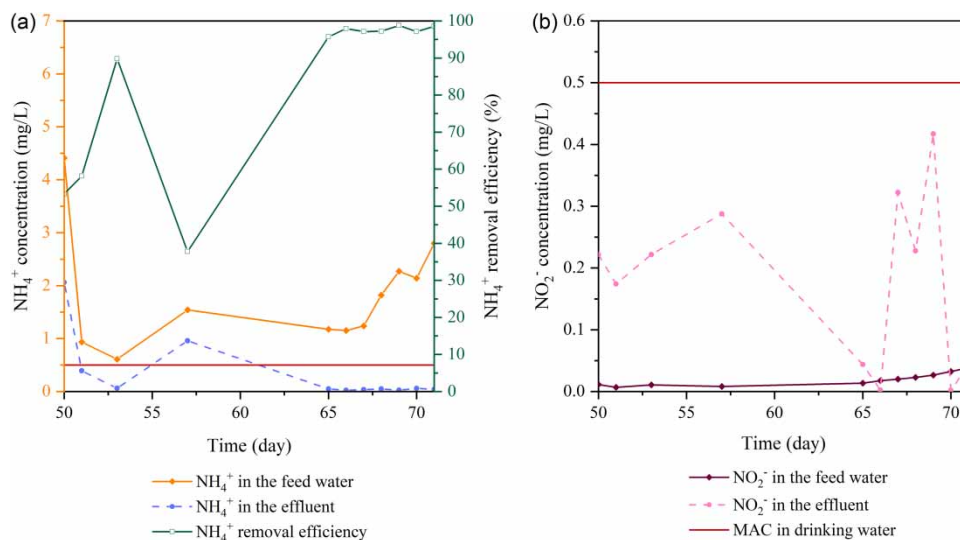


Figure 3 | Incomplete nitrification phase on the quartz sand filter (F_1).

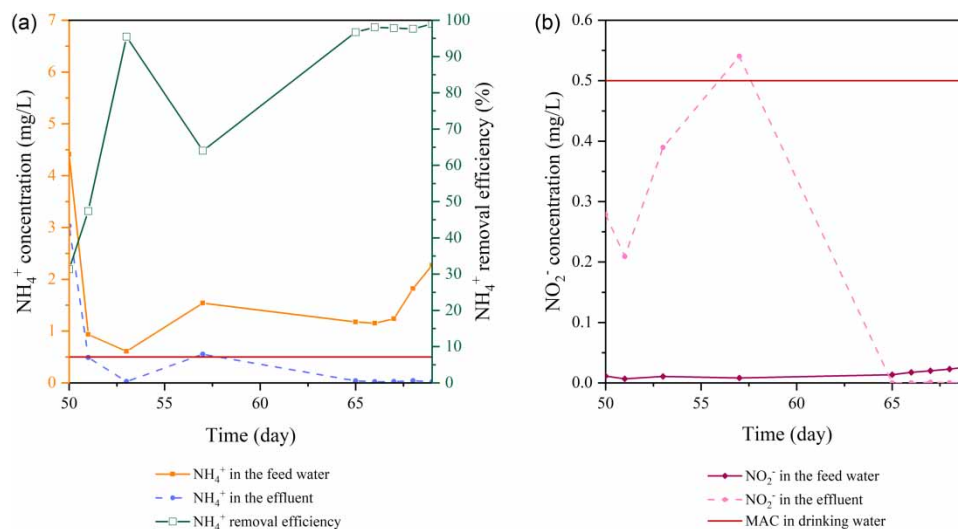


Figure 4 | Incomplete nitrification phase on the quartz sand + anthracite filter (F_2).

nitrification process, it can be concluded that the formation of nitrifying biofilm and the start-up of new biofilters in the biological treatment of drinking water took about 2 months. This was in agreement with many authors who stated in their works that the start-up period usually lasts 1–4 months (Tekerekopoulou *et al.* 2013; Albers *et al.* 2018).

The period of complete nitrification on the quartz sand filter was achieved after 72 days with, until then, the maximum achieved ammonium removal efficiency of 98.8% and nitrite removal efficiency of 96.1%. The obtained results for the quartz sand filter are in agreement with other similar studies, where it is stated that even after enrichment of sand filters with the active nitrifying consortium, it took up from 1 to 3 months before establishing complete nitrification with an ammonium removal efficiency of 98% (Albers *et al.* 2018; Hasan *et al.* 2019).

Complete nitrification on the two-media filter F_2 was established after 65 days. The time difference of 7 days needed for complete nitrification on these two filters was probably related to the fact that they are two different media, different specific surface areas of the filter media particles, which causes different distribution of bacteria on their surface (Kihn *et al.* 2002). However, complete oxidation of NH₄⁺ to NO₃⁻ occurs very quickly on both filters, with a slight difference in removal efficiency (98.8% for F_1 and 96.7% for F_2 , respectively).

The removal efficiency of biofilter F_2 is in agreement with the research conducted on biologically activated filters made of granular activated carbon as a filtration media, where the maximum percentage of NH₄⁺ removal was over 97% (Yapsakli *et al.* 2010). Yapsakli *et al.* (2010) confirmed that up to 92% of ammonium from the influent was removed in the upper layer of the filtration medium, to 0.25 m deep, which ensures the claim that the two-media filter can be viewed as a biofilter made only of biologically activated anthracite.

The mean water temperature measured during the filter start-up period was 12 °C, much lower than the optimal temperature for the AOB and NOB growth (from 25 to 30 °C). Still, it is considered a high enough temperature for complete nitrification (Albers *et al.* 2018). The pH value of the water, which ranged from 7.8 to 8, was optimal for the growth and function of both groups of bacteria (Huyen Le *et al.* 2019).

As one of the most critical process parameters, the filtration rate was maintained at 0.5 m/h in the start-up period, which corresponds to the EBCT of 2.4 h. Drljača *et al.* (2016) stated that complete nitrification could not be achieved when starting the filter at higher filtration rates. In their study, after almost 2 months at high filtration rates (first at 5 m/h and then at 3.3 m/h, which is consistent with EBCT of 0.24 and 0.36 h, respectively), complete nitrification did not occur. When they reduced the filtration rate to 0.8 m/h (EBCT of 1.5 h), complete nitrification took place immediately, followed by the decrease of NH₄⁺ and NO₂⁻ concentrations and the increase of NO₃⁻ concentration in the effluent after filtration.

The period of complete nitrification and the influence of the filtration rate

When complete nitrification on biofilters was established at the pilot plant, several measurements of the dissolved oxygen concentration were done in the feed water and the effluent. Since water aeration during filtration was not performed,

these measurements aimed to determine the oxygen consumption by bacteria for different inlet concentrations of NH_4^+ . This way, the maximum concentration of NH_4^+ that could be oxidised with the present oxygen was determined without the need for prior aeration. The results for oxygen consumption and the amount of oxidised NH_4^+ and NO_2^- are presented in the graph in Supplementary Material.

The theoretical oxygen consumption for the oxidation of 1 mg NH_4^+ /L to NO_3^- is 3.565 mg O_2 (Lytle *et al.* 2007). Concentrations of dissolved oxygen in feed water in this period ranged from 9.14 to 12.24 mg/L, which, according to theory, is sufficient for the oxidation of ammonium in a concentration of 2.5–3.4 mg/L, respectively. During filtration at the pilot plant, oxygen consumption was generally higher. The reason oxygen was consumed in an amount that is higher than theoretically required is that other aerobic bacteria and microorganisms are present in the raw river water, and they also consume oxygen to decompose biodegradable matter (Kihn *et al.* 2002). Based on the results obtained at the pilot plant, it was concluded that the maximum concentration of ammonium that can be oxidised by available oxygen at this pilot plant, without previous aeration, is approximately 2.50 mg NH_4^+ /L.

After the growth of NOB and the increase in the rate of nitrite oxidation, a period of complete nitrification occurred, where ammonium and nitrite were removed entirely, and it can be said that the biofilters were then fully formed. In this period, in addition to the change in the concentration of NH_4^+ and NO_2^- , the change in the nitrate concentration was also measured. The increase in the concentration of NO_3^- in the effluent, compared to the concentration in the feed water, is the result of the complete development of the second stage of nitrification. Nitrate is the final form in which nitrogen is found at the end of this biological process (Huyen Le *et al.* 2019).

Monitoring of the complete nitrification process at the pilot plant was continued only on the two-media filter F_2 (quartz sand + anthracite). The biofilter operating period of the complete nitrification phase, during which measurements were made and nitrification efficiency was examined, lasted 83 days.

Figure 5 shows the results of measuring the concentration of ammonium, nitrite and nitrate in water before and after filtration. The operational conditions as filtration rate and raw water temperature during this period are also given in Figure 5. The complete nitrification phase started on the 65th day after the start of the experiment and was monitored until the 148th day after the filter was put into operation.

From the graph in Figure 5, it is evident that the nitrification process was complete. The exceptions are the measurements taken from day 144 when the raw water temperature was reduced, which will be discussed later. The conclusion that nitrification was

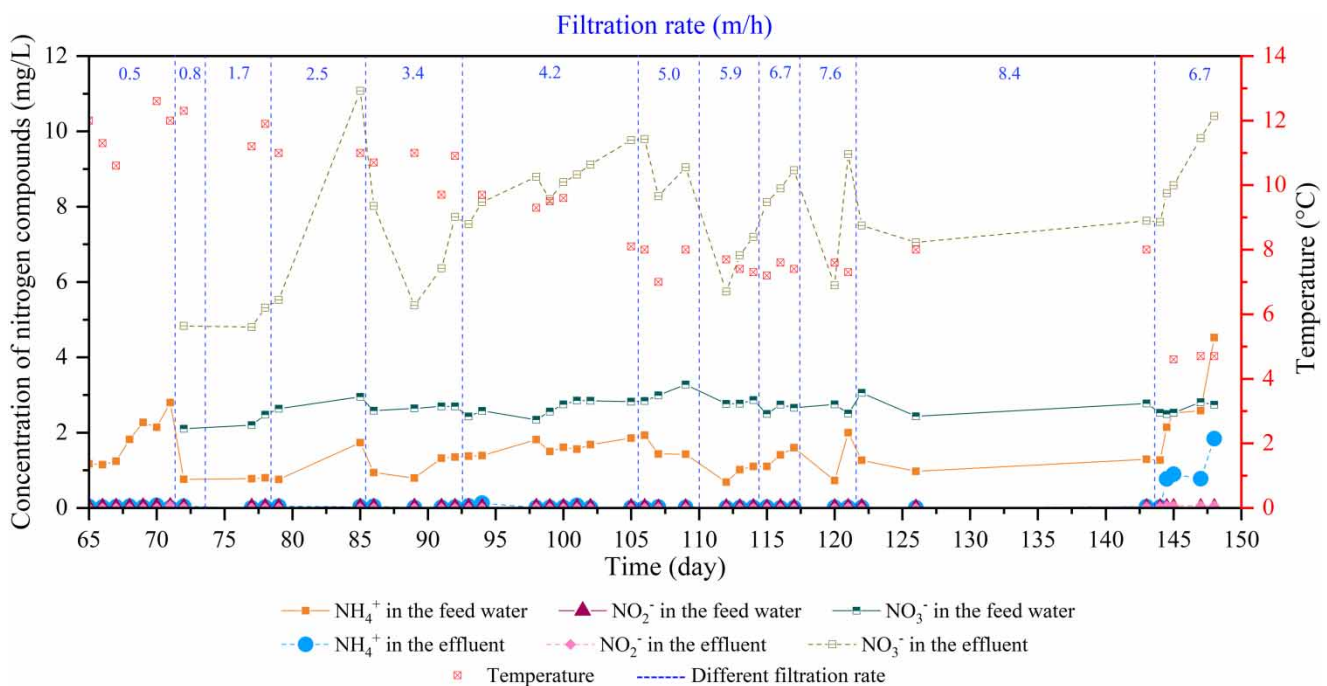


Figure 5 | Complete nitrification phase.

complete in this period can be made based on the decrease in ammonium concentration and no nitrite accumulation in the effluent. Also, everything is followed by an increase in the nitrate concentration in the filtered water. The output concentrations of the measured nitrogen compounds in the water after filtration were below the MAC for drinking water (Sl. Glasnik RS 88/17).

Changes in the inlet concentration of NH_4^+ in this period did not affect the removal efficiency, except for the period when the temperature dropped, after day 144. This is confirmed by the results from day 71 when the concentration of ammonia in the feed water was 2.8 mg/L and the removal efficiency was 99.75% at the temperature of 12 °C. On the other side, on day 147, the ammonia removal percentage was 70% at the concentration of ammonia in the feed water of 2.6 mg/L, but at a much lower temperature of 4.7 °C. Removal of 59.28% on day 148 was influenced by a lower temperature of 4.7 °C, but also a higher inlet concentration of ammonia.

The influence of the filtration rate was examined by monitoring the change in the concentrations of NH_4^+ , NO_2^- and NO_3^- in the effluent for examined filtration rates. The influence of the following filtration rates was tested: 0.5, 0.8, 1.7, 2.5, 3.4, 4.2, 5.0, 5.9, 6.7, 7.6, and 8.4 m/h. These filtration rates correspond to EBCT of 2.4, 1.5, 0.71, 0.48, 0.35, 0.29, 0.24, 0.20, 0.18, 0.16 and 0.14 h, respectively. During this period of the experiment, the raw water temperature decreased from the initial 12.6 to 7.2 °C. Changes in the temperature of raw water can be seen in Figure 5.

Figure 6(a) shows the change in the concentration of ammonium in the feed water and effluent, presented together with the filtration rates maintained at the time of sampling and performed analyses. The dependence of the efficiency of the nitrification, expressed through the NH_4^+ removal percentage, on the filtration rate, is given in Figure 6(b). The NH_4^+ removal percentage for all tested filtration rates was >95%.

The maximum established filtration rate was 8.4 m/h (which matches the EBCT of 0.14 h). This was the maximum filtration rate that could be achieved at the pilot plant. It should be noted that the examined maximum filtration rates were higher than those in the full-scale production plant, which has the capacity to supply drinking water to the city of Banja Luka, which has around 250,000 citizens.

Similar results of high efficiency of nitrification and removal of ammonium at filtration rates of 3.9 and 7.9 m/h were obtained on biological filters made of quartz sand in research conducted in Denmark (Lee *et al.* 2014). The authors of that work found that the removal of ammonium was complete and did not depend on the filtration rates. However, the inlet concentrations of ammonium were lower than in this research, with maximum inlet concentrations up to 0.20 mg NH_4^+ – N/L.

Other numerous studies can be found which indicate that nitrification can be performed on rapid sand filters or biologically activated carbon filters at high filtration rates, with a high percentage of ammonium removal (Hasan *et al.* 2019). Tränckner *et al.* (2008) examined the nitrification on sand biofilters, after inoculation with nitrifying biomass at the maximum filtration rates of 6–7 m/h, and the obtained results of ammonium removal were up to 99%. Also, Suprihatin *et al.* (2017) examined the process of biological filtration on sand filters where, in addition to the removal of ammonium through the nitrification process, they simultaneously monitored the removal of organic matter due to the microbiological activity of heterotrophic bacteria in the drinking water preparation. The process is observed depending on the different hydraulic retention time

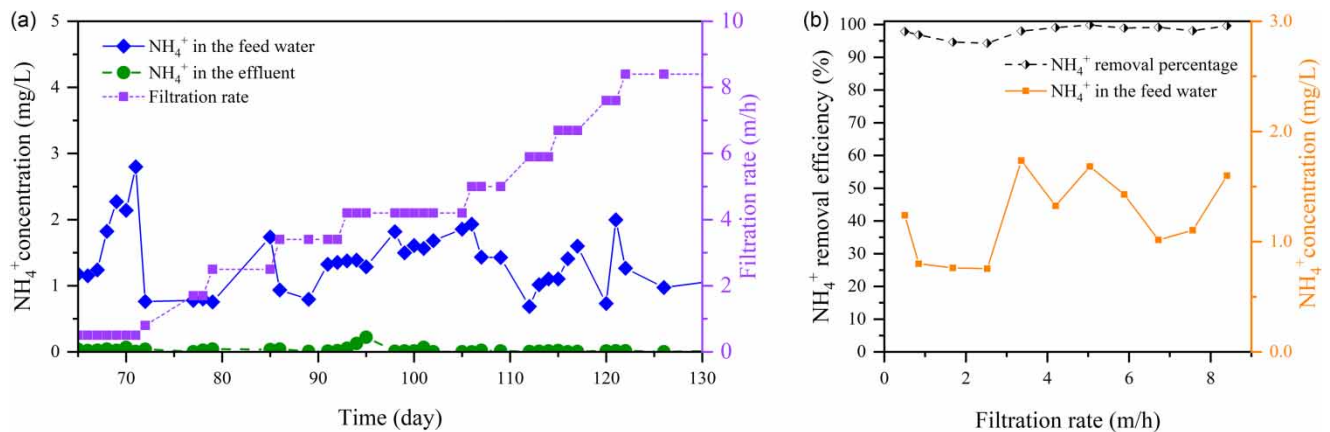


Figure 6 | The influence of filtration rate on the nitrification process: (a) NH_4^+ concentration changes and performed filtration rates and (b) removal percentage of NH_4^+ depending on the filtration rate.

(HRT). Their results show that the percentage of NH_4^+ removal was 90% at HRT of 3 h, but with a decrease in HRT, the removal percentage also decreased. Thus, at an HRT of 0.5 h, the efficiency dropped below 60% (Suprihatin *et al.* 2017).

In this research, the maximum ammonium removal percentage of 99.6% was obtained even at the maximum filtration rate of 8.4 m/h, i.e., EBCT of 0.14 h (Figure 6(b)). It has been proven that the process is effective even at higher filtration rates, which enables the application of the nitrification process in plants for drinking water treatment, in which rapid filters must be used due to the production of large quantities of drinking water.

The influence of temperature on the nitrification process

During the operational period of the biofilter, raw water temperature measurements were carried out daily. Water temperature is an essential factor that affects the activity of nitrifying bacteria and the ammonium oxidation rate (Tekerekopoulou *et al.* 2013; Zhang *et al.* 2014). Therefore, the change in raw water temperature could not be ignored. In the phase of complete nitrification, raw water temperature dropped from 12.6 to 4.6 °C. The influence of water temperature becomes indicative in the days of complete nitrification on F_2 when the water temperature dropped below 5 °C. The values of raw water temperature, with the concentrations of NH_4^+ in the water before and after filtration, are shown in Figure 7(a). Figure 7(b) shows the most frequently measured water temperatures on F_2 . The results of NH_4^+ removal efficiency are given for approximately identical concentrations of NH_4^+ in feed water.

At a filtration rate from 4.2 to 8.4 m/h and an input NH_4^+ concentration of ~1.5 to ~2 mg/L, the raw water temperature was between 7 and 8 °C. The ammonium removal efficiency in this period was high, over 98%. A decrease in efficiency was noticed at the end of January when the raw water temperature dropped below 5 °C. In that period, the biofilm formed on the filter is inhibited, and the oxidation rate decreases. AOB are particularly sensitive to low temperatures (Soliman & Eldyasti 2018). Therefore, incomplete nitrification at lower temperatures is proven by an increase in NH_4^+ concentrations and accumulation of NO_2^- in the effluent.

The exact temperatures at which a drop in nitrification efficiency was registered are 4.6 and 4.7 °C. The removal efficiency at temperatures above 7 °C is over 98%, while at temperatures of 4.7 and 4.6 °C, the NH_4^+ removal efficiency decreases to 70.0 and 63.9%, respectively.

Zhang *et al.* (2014) stated in their research that the oxidation rate decreases significantly at temperatures of 7 °C but achieving a high degree of ammonia removal is still possible. While at temperatures below 5 °C, there is a significant inhibition of nitrifying bacteria activity during biofiltration. However, to make more precise conclusions about the influence of temperature on nitrification efficiency during filtration, it is necessary to conduct experiments under temperature-controlled conditions.

The influence of filter backwashing on the nitrification process

Filter backwashing can have a negative influence on the performance efficiency of biofilters for nitrification (Tekerekopoulou *et al.* 2013).

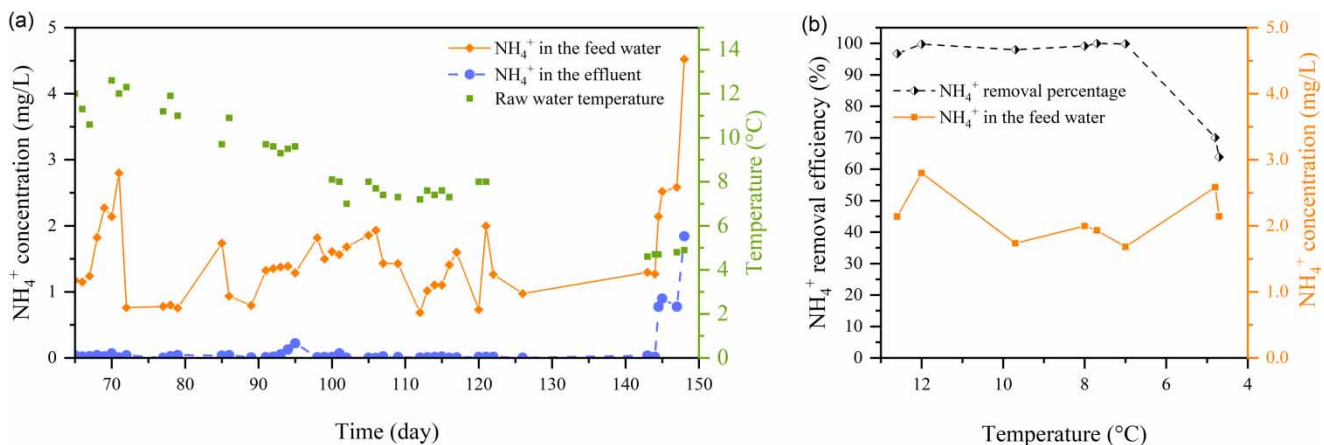


Figure 7 | The influence of temperature on the nitrification process: (a) NH_4^+ concentration changes and measured temperatures and (b) removal percentage of NH_4^+ depending on the temperature.

Table 4 | Results of ammonium ion removal efficiency after filter backwashing

Filter	Day from the start of filtration		Temperature (°C)	Filtration rate (m/h)	EBCT (h)	NH ₄ ⁺ removal efficiency (%)
F ₁	80th	Before backwashing	11	2.5	0.48	98.88
		After backwashing				96.62
F ₂	126th	Before backwashing	8	8.4	0.15	99.75
		After backwashing				98.84

In this work, filter backwashing had to be done because suspended solids accumulated in the filter medium led to decreases of filtration rates which influence was examined in these experiments. Therefore, filter backwashing was performed at times when filtration rates dropped below the set values due to the accumulation of suspended solids from surface water.

Table 4 shows the results of the ammonia removal efficiency after backwashing for each filter on which the nitrifying biofilm was formed. An example of removal efficiency is given for both biofilters, quartz and quartz + anthracite filter, in the case when the sampling of the effluent was performed 2 h after filter backwashing was performed.

Backwashing of the quartz sand filter (F₁) was carried out on day 80 since the beginning of the filtration. During this period, the filtration rate and temperature of raw water were 2.5 m/h and 11 °C, respectively. The efficiency of ammonia removal before backwashing was 99.88%. Two hours after backwashing, for the same operational conditions, ammonia removal efficiency decreased slightly, but still was satisfactory, 96.62% and ammonia concentrations in effluent water were below the MAC for drinking water. Similar results were obtained for dual filter F₂ (quartz + anthracite), but at different operational conditions, what can be seen from Table 4.

CONCLUSIONS

Start-up of nitrification biofilters in the treatment of surface water for biological removal of ammonium and conducted research on the effect of operating conditions at the pilot plant in 'Vodovod' AD Banja Luka led to the following conclusions:

- The spontaneous formation of nitrifying bacteria occurred on the surface layer of filter material as a result of the continuous flow of raw river water with the addition of ammonium.
- The formation of a nitrifying biofilm on the filters was achieved by a continuous flow of water containing ammonium in a concentration ~1 mg/L, dissolved oxygen in a concentration of at least 9 mg/L at a filtration rate <1 m/h and a water temperature of 12 °C up to 13 °C.
- The time required for start-up of the new biofilters and complete nitrification lasted 72 days on the quartz sand filter and 65 days on the quartz sand + anthracite filter.
- For all tested filtration rates at the pilot plant, from 0.5 to 8.4 m/h, a high percentage of ammonium removal (>95%) was achieved at the NH₄⁺ inlet concentration of 0.7 to 2.3 mg/L, without prior aeration and at an inlet water temperature of 7–12 °C.
- Changes in the input concentrations of ammonium (from 0.7 to 2.3 mg/L) did not affect the efficiency of the process and the percentage of removal during filtration after the establishment of complete nitrification.
- The counter-current backwashing of the filter at the pilot plant did not significantly affect the microbiological activity and stability of the biofilm; instead, the nitrification process continued smoothly even after the filter backwashing.

This research showed that the biological removal of ammonium during filtration can be applied in plants for drinking water treatment. The achieved high percentages of NH₄⁺ removal at the maximum performed filtration rates and raw water temperature higher than 7 °C indicate that the process can meet the projected drinking water production capacities.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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