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Reduction of water calcium hardness in the denitrifying biofilter

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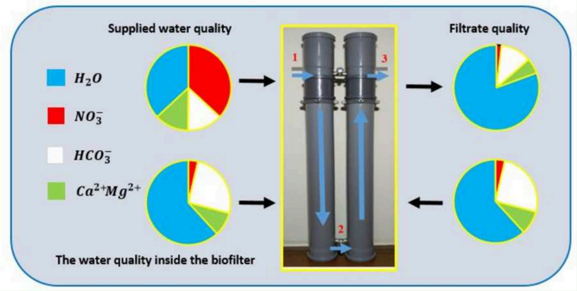
ABSTRACT

Sustainable development goals emphasize the need for cost-effective removal of nitrates from water of sources where there is no centralized tap water supply. This paper describes the properties of the carbonate system in the filtrate of the point of use handy submersible denitrifying biofilter. Device has a U-shaped design with open tops of both elbows and operates in displacement (piston) mode. These allows getting up to 5 liters of denitrified water daily in one gulp, reducing the concentration of nitrate ions in the water by 96-98% from its initial value of $3 \cdot 10^{-3}M — 1.2 \cdot 10^{-2}M$ and the water hardness to reduce depending of initial nitrate concentration. The denitrification process occurs with the consumption of ethyl alcohol as a bacterial food substrate injected to water and accompanies by the accumulation of alkaline bacterial exometabolites in the filtrate. During the microbiological transformation of $3 \cdot 10^{-3}M$ nitrate ions into nitrogen gas, the filtrate enriches with $2.6 \cdot 10^{-3}M$ bicarbonate ions and $3.9 \cdot 10^{-4}M$ of hydroxyl ions. This results in a reduction of filtrate hardness from 3.75 mg-eq/L to 1.87 mg-eq/L when in contact with atmospheric air. Physical experiments and mathematical modeling verified the concentrations of carbonate components and calcium ions in both the initial and denitrified water.



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Denitrification; biofilter; exogenic metabolites; (exometabolites); water hardness; dynamics of hardness reduction

1. Introduction

Water changes its composition in the hydrological cycle. Fresh, soft, and slightly acidic water from atmospheric precipitation becomes mineralized and hard as it percolates through the soil and carbonate rocks to the water-bearing strata and forms underground streams (1). When this water reaches the earth's surface, it becomes softer due to the release of excess carbon dioxide into the atmospheric air and the precipitation of calcium ions (2). Groundwater accumulates nitrates in the regions of intensive agriculture, and where there is no centralized water supply and sewerage. The concentration of nitrate ions in that water is often many times higher than the permissible limit. The WHO guideline value for nitrate in drinking water is 50 mg/L as nitrate ion (equivalent to11 mg/L as nitrate-nitrogen) Nitrate-contaminated hard groundwater requires appropriate conditioning. Methods of ion exchange, electrodialysis, nanofiltration and reverse osmosis currently used to remove nitrates and correct water hardness from problematic underground sources do not meet the criteria of green chemistry and sustainable development goals (environmental friendliness, economy, efficiency). Therefore, the search for other more appropriate methods and approaches continues.

At any given time, the concentrations of protons (pH), calcium cations, dissolved carbon dioxide, carbonic acid, and its dissociation and hydrolysis products (HCO_3^-, CO_3^{2-}) in the carbonate system of water are interrelated (2). From a physical and chemical point of

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view, groundwater with dissolved minerals of carbonate rocks before reaching the earth's surface is a closed carbonate system, and the water that has reached the earth's surface is an open carbonate system (3). The microflora of the biosphere (4) influences the composition and physical properties of groundwater and surface water.

The study of conjugated physicochemical and microbiological processes in the water of natural and model carbonate systems expands the ideas about the peculiarities of formation of their mineral and organic composition. The results obtained reveal the mechanisms of changes in the structure of water associated with the removal of target admixtures and the deposition of hardness salts. This knowledge is necessary to improve the existing technologies of tap water preparation and wastewater treatment, to prevent clogging and corrosion of water pipelines, and to develop new methods of conditioning water consumed from different parts of its natural cycle (5).

Figure 1. Photo of a biofilter and the trajectory of water shifts in it. '**1**', '**2**', '**3**' – points of sampling.

The course of chemical reactions leading to the biologically induced formation of hardness salt precipitation in the water bulk of carbonate systems depends on the degree of accumulation in their alkaline bacterial products (bacterial exometabolites) (6). Alkaliphilic bacteria release ammonia and carbamic acid into water, thus initiating precipitation of calcium ions and other polyvalent cations by increasing pH (7). Denitrifying and sulfate-reducing bacteria in an anaerobic environment (in a closed carbonate system) release bicarbonate and hydroxyl ions into the water (8). This process occurs because of the assimilation of carbon by bacterial cells from nutrient substrates and the use of oxygen from nitrate and sulfate ions to respiration. Hydrolysis of bicarbonate ions results in the appearance of carbonic acid and additional hydroxyl ions (H_2CO_3 and OH^-) in water. As they accumulate, the hydroxyl ions interact with bicarbonate ions to form carbonate ions, which interact with calcium ions to form a calcium carbonate precipitate (8).

Ammonia and carbamic acid cause rapid precipitation of hardness salts in open and closed carbonate systems because the pH of the water there increases in response to the appearance of named exometabolites without the participation of atmospheric air. The situation is different for the accumulation of bicarbonate ions. In a closed carbonate system, the appearance of an additional amount of bicarbonate ions (due to injection from outside or due to microbiological activity within the system) does not increase pH. In this case, the hydrolysis of bicarbonate ions is inhibited by the carbonic acid accumulating in the water. As a result, the concentration of carbonate ions required to form calcium carbonate precipitate remains below the solubility product. However, suppose the carbonate system gains access to the atmospheric air. In that case, the equilibrium of the hydrolysis reaction of hydrogen carbonate ions shifts to the right due to the volatilization of carbon dioxide from carbonic acid. As a result, the accumulation of hydroxyl and carbonate ions in the water facilitates. These ions ensure the formation of calcium carbonate sediments and mixed calcium-magnesium carbonates when free calcium and magnesium cations are available in the water. The most significant changes in component concentrations in the carbonate system occur during intense gas exchange.

Bacterial excretions into the water of carbonate systems in technical devices – biological reactors and biological filters one can use for additional softening of treated water (7, 9–11).

This work aimed to reveal the regularities of water hardness change in a small-sized submersible denitrifying biofilter (point of use). The object of study was initial (supplied) water and filtrate from the device, which operates in the mode of displacement (piston) filtration (12) and reduces the concentration of nitrate ions in water by 96-98% from the initial level of.

$3 \cdot 10^{-3}M - 1, 2 \cdot 10^{-2}M$. The magnitudes of pH and pH-related concentrations of bicarbonate ions, carbonate ions, calcium ions, dissolved carbon dioxide, and dissolved carbonic acid in water at the inlet of the biofilter and in the filtrate sampled from the inside of the biofilter and at its outlet were the subject of study.

2. Experimental part and theoretical basis

2.1. Materials and methods

This work investigated pH and hardness changes along the trajectory of water shifts in a small-size submersible denitrifying biofilter Point of use. The photo of it is in Figure 1. The design of the biofilter, its mode of the run, and denitrifying efficiency are described in (12, 13). The device denitrifies water in the mode of displacement filtration for five years of continuous operation on a bench scale. During this time, two and a half up to five liters of tap water with added sodium nitrate supplied daily (in one gulp) to the inlet of the biofilter so that the initial nitrate concentration is $3, 0 \cdot 10^{-3}M$ and the same amount of denitrified water flows out of the biofilter. The nutrient substrate (ethanol) in the feed water portion dosed for 0.079 g/L ($\sim 1.7 \cdot 10^{-3}$ M) as it is required according to the stoichiometry of the reaction of the biological reduction of nitrate nitrogen to molecular nitrogen (14). Ethanol ensures required conversion of nitrate ions into molecular nitrogen without the accumulation of toxic intermediates in the filtrate (14). Residual ethanol not detected in the water at biofilter exit (15). The concentration of nitrate ions in the synchronously obtaining filtrate does not exceed a few milligrams per liter at a temperature of 20-23°C, if the portions of supplied water are below of five liters. Hydraulic retention time of supplied water is 3 days for 5L portions and 6 days for 2.5L portions (12, 13).

Blue arrows in the photo show the direction of shifting of portions of denitrified water inside the biofilter. Water for pH metric and chemical-analytical studies of 130 and 50 mL, respectively, were sampled from the inlet compartment (**1**) in the inlet elbow of the biofilter, the conjunction zone (**2**) of the inlet and outlet elbows of the biofilter, and of the outlet compartment (**3**) in the outlet elbow. The cell for pH metrology was a standard 150 mL chemical beaker with a magnetic stirrer and a miniature atmospheric air sprayer. The air sprayer was immersed to a depth of 20 mm from the surface of the water sample in the beaker. A flow of air bubbles was used with air discharge of 0.9 L/min and air bubbles radius 0.5-1.5 mm. Dynamics of pH change in water samples (volume – 130 mL) was recorded automatically by pH meter – ion-meter I-160MI, equipped with glass pH electrode ESC 10603 and chloride-silver reference electrode EVL1M3.1. The pH measurement results were recorded according to the set algorithm every 20 sec during the measurement time up to 15,000 sec. Was used a personal computer with the software 'Analytics' installed on it. The concentration of calcium and magnesium ions in water samples was determined by the standard titration method using Disodium EDTA.

2.2. An explanation of the context and outcomes of mathematical modeling

In any aquatic calcium – containing carbonate system (16), the concentrations of protons (pH), calcium cations, dissolved carbon dioxide, carbonic acid, and its dissociation and hydrolysis products (anions- HCO_3^- , CO_3^{2-}) are interrelated with each other. System pH serves as a significant parameter and useful indicator of the water state, particularly of its temporary hardness. Methods for softening hard water using caustic soda, slaked lime, and soda ash rely on alkaline pH shifts.

The alkaline activity of certain bacteria, such as Corynebacterium ammoniagenes (17), Sporosarcina pasteurii (18), and Bacillus subtilis (19), increases pH in carbonate systems. These bacteria thrive in specific niches found in soil, water bodies, and the zoo sphere. By secreting alkaline exometabolites, Alkaliphilic bacteria regulate the concentrations of calcium ions, magnesium ions, and others that contribute to water hardness (7). When these microbes absorb arginine or urea, they produce ammonia and carbamic acid. In the case of urea absorption, the reaction is (20):

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3. \qquad (1)$$

The hydrolysis of carbamic acid results in the release of additional ammonia and the formation of carbonic acid in the water:

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3. \qquad (2)$$

Dissolving ammonia produces ammonium hydroxide, which dissociates into ammonium ions and hydroxide ions according to the equation:

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4OH \leftrightarrow 2NH_4^+ + 2OH^-. \qquad (3)$$

Here, the coefficient 2 is in front of the symbols for the initial substances and reaction products. This is because both (Equation (1)) and (Equation (2)) contribute to the formation of ammonia. Due to the dissociation of carbonic acid (see Equation (2)), bicarbonate ions and protons appear in the water via the reaction:

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+. \qquad (4)$$

Additionally, carbonic acid partly decomposes to form carbon dioxide and water:

$$H_2CO_3 \leftrightarrow H_2O + CO_2. \uparrow \qquad (5)$$

The reaction between HCO_3^- and $H^+$with the hydroxyl ions produced by Equation (3) yields carbonate ions:

$$HCO_3^- + \ H^+ + 2OH^- \ \leftrightarrow \ CO_3^{2-} + 2H_2O \qquad (6)$$

These carbonate ions combine with ammonium ions to form ammonium carbonate:

$$2NH_4^+ + CO_3^{2-} = \ (NH_4)_2CO_3 \qquad (7)$$

The equation summarizes the resultant chemical reaction:

$$CO(NH_2)_2 + 2H_2O \rightarrow (NH_4)_2CO_3 \qquad (8).$$

Ammonium carbonate is a salt composed of a weak acid and a weak base, resulting in a pH of 9.19 (C = 2 mM, t = 25°C). At this pH, the temporary calcium hardness decreases to 0.1 mg-eq/L.

The chemical reaction that produces calcium carbonate from calcium bicarbonate and ammonium carbonate is as follows:

$$Ca(HCO_3)_2 + (NH_4)_2CO_3 \ \rightarrow \ CaCO_3 \downarrow + 2NH_4HCO_3 \quad (9)$$

Alkaliphilic bacteria reduce both temporary and permanent calcium hardness in the water. For example,

$$CaCl_2 + (NH_4)_2CO_3 \rightarrow CaCO_3 \downarrow \ +2NH_4Cl \qquad (10)$$

The ammonium chloride released by the reaction Equation (10) forms a buffer mixture with ammonia. At the equimolar ratio of the components of the buffer mixture, its pH = 9.24. As a result, calcite precipitation occurs in carbonate systems with Alkaliphilic bacteria. Particularly favorable conditions for calcite formation occur when the buffering capacity of the aqueous medium is low. In this case, even small amounts of alkaline exometabolites can shift the pH to nine and higher.

Denitrifying and sulfate-reducing bacteria are also able to decrease water hardness with the formation of calcium carbonate sediments. However, these bacteria do it in other way (8). Under anoxic conditions, denitrifying bacteria split oxygen from nitrate ions for respiration. They use a suitable digestible carbon and energy source as a nutrient substrate. When fed with ethanol (12,14) colonies of denitrifying bacteria produce 0.87 M bicarbonate ions and 0.13 M hydroxide ions per mole of nitrate ions consumed, as shown in the equation:

$$NO_3^- + 0,55C_2H_5OH \ + 0,137O_2 \rightarrow$$
$$\rightarrow 0,046C_5H_7O_2N + 0,87HCO_3^- + 0.99H_2O + 0,13OH^- + 0,477N_2 \qquad (11)$$

When the food substrate for denitrifying bacteria is their biomass ($C_5H_7O_2N$), as studied in (21) the reaction is:

$$NO_3^- + \ \ 0.25\ C_5H_7O_2N \rightarrow 1.25\ \ CO_2 + 0.25\ NH_3 + OH^- + 0.5\ N_2 \qquad (12)$$

Or:

$$NO_3^- + \ \ 0.25\ C_5H_7O_2N + 0,25H_2O \rightarrow HCO_3^- + 0.25\ NH_4HCO_3 + 0.5\ N_2 \qquad (13)$$

In this case, each mole of nitrate ions consumed releases one mole of bicarbonate ions and twenty-five hundredths of a mole of ammonium bicarbonate. Denitrification by bacteria that consume their biomass is a forced process and is much slower than with ethanol feeding.

Bicarbonate ions are also exogenous metabolites in sulfate-reducing bacteria. Under anaerobic conditions, these bacteria split oxygen from sulfate ions for respiration. When ethyl alcohol as a carbon and energy source is consumed (15, 22), the equation for bacterial sulfate reduction is:

$$3SO_4^{2-} + 2CH_3CH_2OH = 3HS^- \ + 3H_2O + CO_2 + 3\ HCO_3^- \qquad (14)$$

For each mole of sulfate ions consumed, one mole of bicarbonate and one mole of hydrosulfide ions appear in the water. Sulfate reduction occurs in denitrifying systems when a nutrient substrate is in excess (15).

The bicarbonate ions produced by biological denitrification and sulfate reduction undergo hydrolysis to form carbonic acid and hydroxide ions as shown below:

$$HCO_3^- + H_2O \ \leftrightarrow H_2CO_3 + OH^- \qquad (15)$$

The equilibrium of the hydrolysis reaction shifts to the left when excess H_2CO_3 or OH^-. Therefore, bicarbonate ions and hydroxyl ions interact first in closed (isolated) carbonate systems. The reaction results in the formation of carbonate ions and water molecules as shown in Equation (6).

The hydrolysis constant of the carbonate ions and the corresponding dissociation constant of the carbonic acid obey the relationships:

$$K_H = [H_2CO_3][OH^-]/[HCO_3^-], \tag{16}$$

$$K_H = \frac{[H_2CO_3][OH^-][H^+]}{[HCO_3^-][H^+]} = \frac{K_W}{K_A} = \frac{(\alpha^*)^2(C_{HCO_3^-})^2}{C_{HCO_3^-}(1-\alpha^*)} = \frac{10^{-14}}{10^{-6,4}} = 10^{-7,6} \tag{17}$$

Here: K_H is the hydrolysis constant of hydrogen carbonate ions, K_W is the ionic product of water, K_A is the first step dissociation constant of carbonic acid, α^* is the degree of hydrolysis, $C_{HCO_3^-}(1-\alpha^*)$ is the concentration of hydrogen carbonate ions that have not undergone hydrolysis. $\alpha^*(C_{HCO_3^-})$ is the concentration of hydrogen carbonate ions that give rise to equivalent concentrations of H_2CO_3 and OH^-.

In (Equations (16) and (17)) concentrations are used instead of activities, because at not high content of dissolved salts in water (< 2 g/dm^3) the ionic strength is low, and the activity coefficients can be considered equal to one. Knowing also that the degree of hydrolysis of carbonic acid anions (α^*) is substantially less than unity, $(1-\alpha^*) \approx 1$) one obtains.

$$\alpha^* = \sqrt{\frac{K_W}{K_A C_{HCO_3^-}}} = \sqrt{\frac{K_H}{C_{HCO_3^-}}} \tag{18}$$

Given that:

$$[OH^-] = \alpha^* C_{HCO_3^-}\sqrt{K_H \cdot C_{HCO_3^-}} = \tag{19}$$

One got:

$$pH = 14 + lg\sqrt{K_H \cdot / C_{HCO_3^-}} \tag{20}$$

Using (Equations (20), (18), and (11)), one can evaluate possible shifts in the pH of the filtrate due to the accumulation of HCO_3^- in it. Particularly, if biological denitrification results in the removal of $3 \cdot 10^{-3}M$ nitrate ions (186 mg/L in terms ofNO_3^-) from the water, then $2, 6 \cdot 10^{-3}M$ hydrogen carbonate ions will appear. The degree of hydrolysis of these ions and the pH determined by (Equations (18) and (20)) should be 0.0031 and 8.91, respectively. However, the obtained results are right describing the carbonate system only when it is in contact with atmospheric air (the system is open), and no cations are present that form insoluble precipitates with hydroxyl ions and carbonate ions (i.e.when the carbonate system is incomplete in relation to calcium ions). When this condition is met, the hydrolysis equilibrium according to (Equation (15)) shifts to the right (carbon dioxide escapes to the atmosphere) and hydroxyl ions accumulate in the water. Their interaction with bicarbonate ions ensures the appearance and accumulation of carbonate ions:

$$HCO_3^- + OH^- \Leftrightarrow CO_3^{2-} + H_2O \tag{21}$$

Thus, based on (Equations (11), (12), (15), and (21)), one obtains:

Open carbonate system: $H_2O + CO_2 \uparrow$ Carbon dioxide volatilize from the water.

$$2HCO_3^- + H_2O \leftrightarrow H_2CO_3 + \overset{\uparrow\downarrow}{\underset{\uparrow\downarrow}{OH^-}} + HCO_3^- \leftrightarrow CO_3^{2-} + H_2O \tag{22}$$

Closed carbonate system: $H_2O + CO_2 \downarrow$ Carbon dioxide dissolve in the water.

According to (Equation (22)), in the water bulk of a carbonate system, equilibrium is established between the concentrations of bicarbonate ions, hydroxyl ions, carbonate ions, and carbonic acid, depending equilibrium of carbon dioxide between the water and gas phases. Carbon dioxide can be part of the carbonic acid, can dissolve in water as a gas, and can volatilize from water into the atmosphere.

In a closed carbonate system, the water is isolated from atmospheric air (it exists inside a filter load or hermetically sealed body of a biological reactor). The accumulation of bicarbonate ions in this system causes the concentration of carbonic acid and hydrogen ions to increase according to (Equation (4)). The concentration of carbonic acid increases more strongly compared to the concentration of hydrogen ions, as the equation shows:

$$\frac{[HCO_3^-]}{K_A} = \frac{[H_2CO_3]}{[H^+]} \tag{23}$$

According to (Equation (23)), a tenfold increase in the concentration of bicarbonate ions should correspond to a tenfold increase in the concentration of hydrogen ions and a hundredfold increase in the concentration of carbonic acid. Therefore, the addition of bicarbonate ions to the water of a closed (isolated) calcium carbonate system will not lead to an increase in its pH, since the associated accumulation of carbonic acid will shift the equilibrium of the hydrolysis reaction of bicarbonate ions to the left, according to the Le Chatelier principle. The shifted equilibrium 'preserves' the bicarbonate ions by an excess of carbonic acid (see Equation (15)) and prevents their conversion to carbonate ions according to (Equation (22)). As a result, the concentration of carbonate ions remains below necessary for the formation of hardness salt precipitates.

Upon contact with atmospheric air, the equilibrium in the carbonate system saturated with bicarbonate ions shifts toward the accumulation of hydroxyl ions and carbonate ions. As a result, the pH and calcium concentration (temporary hardness of water) shift to equilibrium values, which are predetermined by the concentration (partial pressure) of carbon dioxide in atmospheric air. In reference (2), it was shown that in an open carbonate system, the equilibrium pH = 8.36 when the concentration of CO_2 in atmospheric air is 316 ppm. If the equilibrium pH exceeds this value under the presented conditions, then the carbonate system is most likely incomplete with the content of calcium ions in it. In such a system, bicarbonate anions and their hydrolysis products form perfectly soluble salts and alkalis by combining with alkali metal cations.

However, when calcium ions are added to such a system in required quantities, insoluble calcium carbonate is formed according equation.

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \downarrow \tag{24}$$

As a result, the pH returns to its natural equilibrium value.

Figure 2 shows the calculated plots (black lines) of the decimal logarithms of the equilibrium concentrations of HCO_3^-, Ca^{2+}, CO_3^{2-}ions, and total dissolved carbon dioxide and carbonic acid ($CO_2 + H_2CO_3$) concentrations as a function of pH in the open carbonate system. Calculations were performed according to (2) for the range of physically realizable carbon dioxide concentrations in the air.

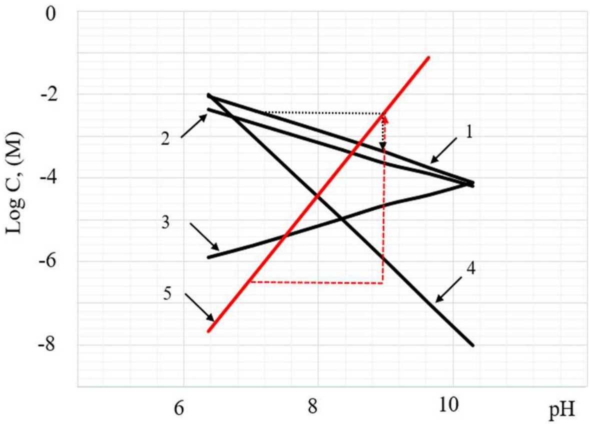

Figure 2. Concentrations of HCO_3^-(Line (1)), Ca^{2+} (Line(2)), CO_3^{2-}(Line(3)), $\sum CO_2 + H_2CO_3$(Line(4))*vs* pH in the water of the carbonate system, balanced by the concentration of carbon dioxide in the air. (Line (5)) shows the effect of the concentration of bicarbonate ions on pH when there are no calcium cations in the water and there is equilibrium on $[CO_2 + H_2CO_3]$ with carbon dioxide in the atmospheric air.

The following relationships between the components of the carbonate system used in calculations:

$$[CO_2 + H_2CO_3] = K_{Henry} \cdot p_{CO_2} \tag{25}$$

$$[HCO_3^-] = \frac{K_{H_2CO_3} \cdot [CO_2 + H_2CO_3]}{[H^+]} \tag{26}$$

$$[CO_3^{2-}] = \frac{K_{HCO_3^-} \cdot [HCO_3^-]}{[H^+]} \tag{27}$$

$$[OH^-] = \frac{10^{-14}}{[H^+]} \tag{28}$$

$$[Ca^{2+}] = \frac{SP_{CaCO_3}}{[CO_3^{2-}]} = \frac{SP_{CaCO_3}[H^+]}{K_{HCO_3^-}[HCO_3^-]} = \frac{SP_{CaCO_3}[H^+]^2}{K_{HCO_3^-} \cdot K_{H_2CO_3} \cdot K_{Henry} \cdot p_{CO_2}} \tag{29}$$

$$2[Ca^{2+}] + [H^+] = 2[CO_3^{2-}] + [HCO_3^-] + [OH^-] \tag{30}$$

Here: SP_{CaCO_3} is solubility product of $CaCO_3$

In an open calcium-containing carbonate system, the equilibrium concentrations of HCO_3^-, Ca^{2+}, CO_3^{2-} ions, are related to the hydrogen ion concentration (pH) through the concentration (activity) of dissolved CO_2 and H_2CO_3 in the water. The latter relies on the concentration of CO_2 in the air, as per (Equation (25)). As the concentration of CO_2in the air increases, the equilibria shift to the left. High concentrations of hydrogen ions correspond to high concentrations of hydrogen carbonate ions (Line (1)), calcium ions (Line (2)), dissolved carbon dioxide, and dissolved carbonic acid (Line (4)). The concentration of carbonate ions (Line (3)) is initially low, but as the pH shifts to the alkaline range, it increases exponentially. In the pH from 6.36–10.29, the bicarbonate and calcium ions concentration decreases by two orders of magnitude, and the concentration of dissolved carbon dioxide and carbonic acid decreases by six orders of magnitude, as (Line(4)) shows. At the same time, the concentration of carbonate ions (see Line (3)) increases by two orders of magnitude. The sums of the logarithms of the concentration of carbonate ions and the logarithms of the concentration of calcium ions at any pH in the designated pH range are numerically equal to the logarithms of the product of the solubility of calcium carbonate.

In a closed calcium-containing carbonate system, equilibria shift under the influence of internal factors. Exometabolites of Alkaliphilic bacteria, i.e. ammonia, and hydroxyl ions change the concentrations of HCO_3^-, Ca^{2+}, CO_3^{2-}due to synchronous decrease in hydrogen ion concentration. As the pH changes from the low equilibrium value to the high one, the concentration of bicarbonate and calcium ions decreases. This is due to the formation of calcium carbonate, which precipitates. The transition shown by the black dashed line, and black arrow in Figure 2.

However, if the internal disturbance is bicarbonate ions, then the effect of their accumulation one see, when the carbonate system becomes open. Due to system opening, the equilibrium of the hydrolysis reaction of bicarbonate ions shifts toward the accumulation of hydroxyl ions and carbonate ions, as shown by (Equations (15) and (22)). The subsequent properties of the system depend on the concentration of 'free' calcium ions. If there are enough calcium ions to bind all the hydrolysis products of bicarbonate ions into insoluble carbonate, then reach an equilibrium pH that is inherent to complete (calcium-containing) system at a given partial pressure of carbon dioxide in atmospheric air. If there are no free calcium ions, but alkali metal cations instead, then establishes an equilibrium pH that is inherent to an incomplete (calcium-free) carbonate system at the same partial pressure of carbon dioxide in atmospheric air. The red solid line in the Figure 2 shows the relationship between the concentration of bicarbonate ions and the pH in such a system. For this case (Equation (20)) was used to perform the calculation. In calcium-free carbonate system, the angular coefficient of the logarithm of the concentration of bicarbonate ions vs. pH has a positive value. At $pH = 6.36$, the concentration of bicarbonate ions is minimal, and at $pH = 10.29$ it is maximal. To shift an open calcium-free carbonate system from $pH = 7$ to $pH = 9$, a significant accumulation of bicarbonate ions is required, surpassing their equilibrium concentration in an open calcium-containing system. This one can see in Figure 2, where the red dotted line represents the transition. The difference in concentrations determines the potential for excess hydrogen carbonate ions to bind additional calcium ions in water when it acquire contact with atmospheric air. Additional calcium ions may be present in the water as a component of its permanent hardness or added to the water as the corresponding salt. The transformation of bicarbonate ions into carbonate ions with the participation of hydroxyl ions, as shown in (Equation (21)), provides the reaction. It is also possible to reveal the dynamics of water hardness change by the results of pH measurement when chemical transformations follow the chain:

(bicarbonate ions) → (carbonate ions plus calcium ions) → (calcium carbonate).

2.3. Results of physic-chemical experiments and discussion

Figure 3 shows the results of the bench experiments. The pH were measured vs time in samples of fed water and water samples collected from the conjunction zone of the inlet and outlet elbows of the biofilter, as well as from the filtrate at the bio filter's exit. Each sample of 130 mL underwent aerating with a stream of atmospheric air bubbles, at the flow rates and bubble radii specified in the Materials and Methods section. Anhydrous calcium chloride was added to the water samples at the times marked by the red dashed arrows in the graphs. The amount of calcium chloride added exceeded the requirement to bind calcium ions to carbonate ions, as per (Equations (11), (21), and (24)). Carbonate ions appear and saturate the filtrate due to the hydrolysis of bicarbonate ions (exometabolites of denitrifying bacteria).

Results show the carbonate system in the source water (Line (1)) in Figure 3(A) is in equilibrium with atmospheric carbon dioxide. The pH in this water does not change practically when it blows by a stream of atmospheric air bubbles. In this, water no excess of bicarbonate ions, which could enrich it with carbonate and hydroxyl ions due to aeration-induced hydrolysis.

The water sampled from the conjugation zone of the inlet and outlet elbows of the biofilter (as shown by the course of (Line (2)) in Figure 3A) contains an excess of bicarbonate ions, and calcium ions deficiency. When air bubbles blows through this water, the carbonate system reaches a new equilibrium state with an increased pH of 8.65-8.72. The transition time lasts for about an hour. Upon adding calcium chloride to this sample, the pH rapidly drops to 8.3-8.2 and calcium carbonate precipitate appears. This suggests that an excess of bicarbonate ions, which are bacterial exometabolites, undergo hydrolysis during aeration to form carbonate ions according to (Equation (22)) and then combine with calcium ions according to (Equation (24)). Prior the time of adding calcium chloride to the sample, it has deficit calcium ions to resist the increase of pH when the sample blew with a stream of atmospheric air bubbles.

Other hand, if calcium chloride adds initially to the water sample taken from the conjugation zone of the biofilter inlet and outlet elbows, the pH changes as shown by the course of (Line (3)). It is due to an excess of calcium ions in the sample, which, resulting of aeration-induced hydrolysis of the initial excess of bicarbonate ions, binds to the formed carbonate ions and leads to a calcium carbonate precipitate. Such a carbonate system returns with time to its natural equilibrium

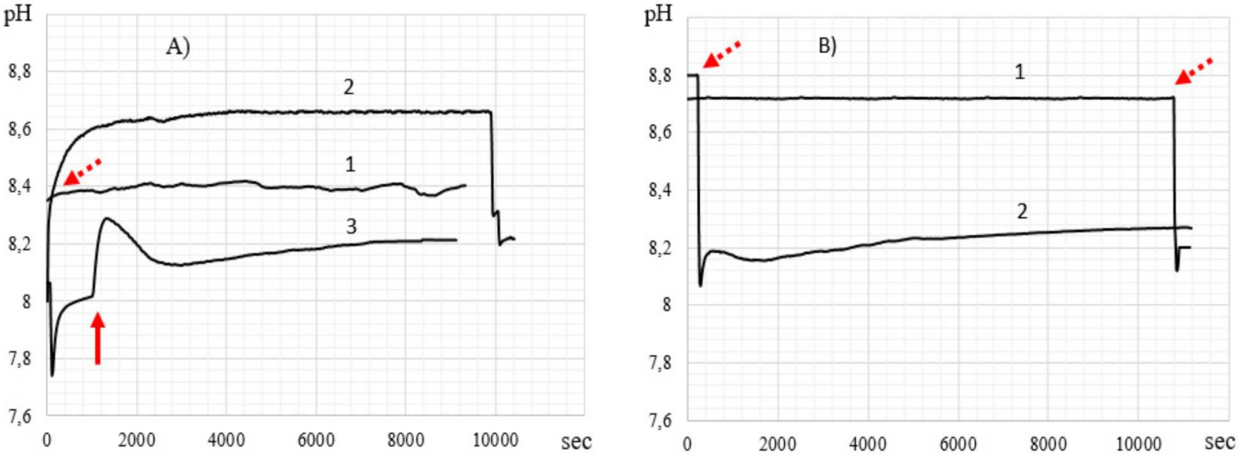

Figure 3. (**A**). The pH change vs. time in the aerating sample of source water (Line (1)). In the aerating sample of water from the interface zone of the inlet and outlet elbows of the biofilter (Line (2)), and in the aerating sample of water from the interface zone of the inlet and outlet elbows of the biofilter (Line (3)) with added calcium chloride. The red solid arrow depicted below (Line (3)) is the moment of starting the blowing of the sample by the flow of air bubbles**.** (**B**). Dynamics of pH change in aerating filtrate (sample from the exit of biofilter) as a function of adding calcium chloride. Red dotted arrows depict the moments of calcium chloride added.

state determined by the partial pressure of carbon dioxide in atmospheric air.

Schematic statement of bicarbonate ions appearing and vanishing zones in created biofilter represented in Figure 4. This is consistent with the results of pH measures at prolonged aeration of water samples taken from the biofilter outlet when calcium chloride is injected into water at the start and the end of the experimental period. The curve of (Line (1)) in Figure 3B shows that the filtrate initially has an excess of bicarbonate ions and a deficit of calcium ions. The carbonate system maintain this state until additional calcium ions are added in the form of calcium chloride. The course of (Line (2)) in Figure 3B leads to the conclusion that with an initial excess of calcium ions in the sample under investigation, the carbonate system in this sample gradually returns to the equilibrium state predetermined by the concentration of carbon dioxide in the atmospheric air.

Bicarbonate ions excess appear in the inlet zone of the biofilter because of biological denitrification

Bicarbonate ions excess remain in their native state inside the biofilter

Bicarbonate ions undergo irreversible hydrolysis at the outlet of the biofilter and thereby reduce calcium hardness in the filtrate due to the interaction of arisen carbonate ions with free calcium ions.

Figure 4. Schematic statement of free (in excess) bicarbonate ions appearing and vanishing zones in the created biofilter.

Chemical analysis showed that the hardness of the water sent to the biofilter was 3.75mg-eq/L, the hardness of the unaerated filtrate remained approximately the same, and the hardness of the aerated filtrate decreased to 1.87mg-eq/L.

The reduced hardness in the aerated water at the biofilter outlet is seems the result of the interaction of carbonate ions (arising in filtrate) with calcium ions that were initially present in the water as a component of its permanent hardness.

Additional information is provided by the results of pH vs time in aerated solutions obtained by sequentially adding anhydrous calcium chloride (0.017 g) and sodium bicarbonate (0.033 g) to 130 mL of distilled water. The molar concentrations of these substances in the mixture were respectively equal to $1.2 \cdot 10^{-3}$ M and $3.0 \cdot 10^{-3}$ M.

Figure 5A shows the pH versus time in distilled water when sodium bicarbonate and calcium chloride (black curve) and vice versa (red curve) were added sequentially. In both cases, sodium bicarbonate was added more than the amount required by stoichiometry to convert calcium chloride to calcium carbonate according to (Equations (15), (21), (24)), i.e.:

$$2NaHCO_3 + CaCl_2 = CaCO_3 \downarrow + (H_2O + CO_2 \uparrow) + 2NaCl \quad (31)$$

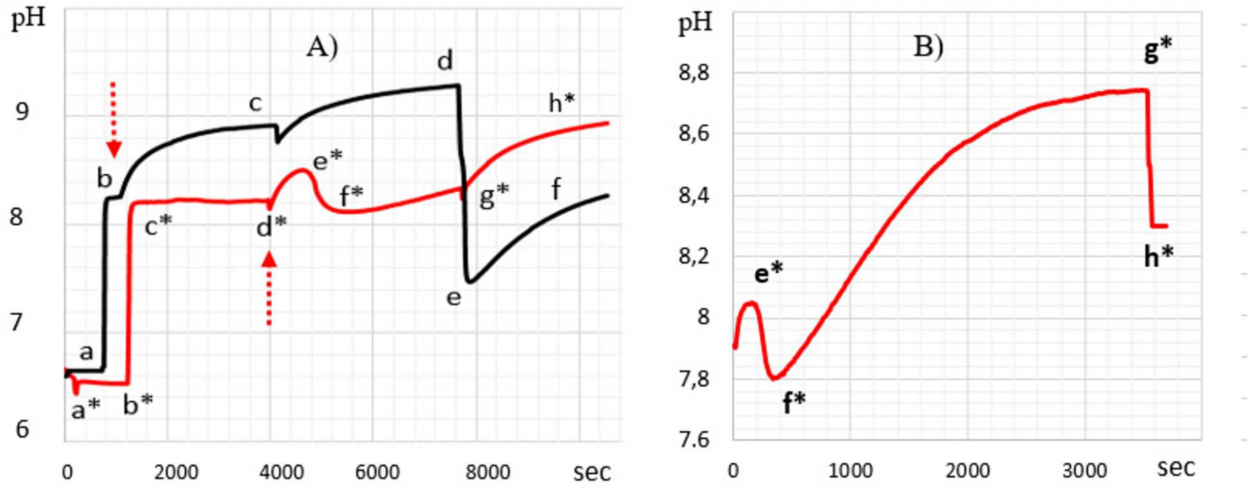

Figure 5. (**A**). Dynamics of pH changes in distilled water (black curve) in response to the sequential add of a handing of sodium bicarbonate (Point (a)), a stream of air bubbles (Point (b)), a second handing of sodium bicarbonate (Point (c)), a handing of calcium chloride (Point (d)), and a handing of sodium bicarbonate (Point (e)). Dynamics of pH change in distilled water (red curve) in response to sequential adding of calcium chloride (Point (a*)), sodium bicarbonate (Point (b*)), air bubble stream (Point (d*)), second handing of sodium bicarbonate (Point (g*)) with continued blowing of the solution by air bubble stream. (**B**). Dynamics of pH changes in the freshly prepared solution of a mixture of calcium chloride and sodium bicarbonate at its excess under the influence of air bubble flow and subsequent addition of calcium chloride at (Point (g*)).

Forced blowing of the formed solutions by air bubble flow was started at the times indicated by the red dashed arrows. Additional amounts of sodium bicarbonate were added to the solutions at (Points (c), (e), and (g*)). Calcium chloride were added to the solutions at (Points (a*) and (d)).

Figure 5B shows the pH vs. time in a freshly prepared and forced aerated solution of calcium chloride and sodium bicarbonate mixture. The excess of sodium bicarbonate in this solution compared to the quantity required for calcium carbonate formation according to (Equation (31)) leads to the same pattern of pH changes over time as of pH metrics in the aerated solution obtained by adding sodium bicarbonate to the calcium chloride, as shown by the red graph in Figure 5A.

The presence of calcium chloride, a salt of strong base and a strong acid, in distilled water, does not cause a significant change in the pH of the resulting solution. This is shown by the (a*)-(b*) plot on the red curve in Figure 5A. The pH quickly rises to a new level with the subsequent addition of 0.033 g of sodium bicarbonate to the solution, which is more than the required mass for the reaction to proceed according to (Equation (31)). The new pH is not shifts essentially until turned on a stream of air bubbles into the solution. This is shown by the graph (c*)-(d*). Then the pH change occurs along the trajectory (d*-e*-f*-g*) and after the addition of a new portion of sodium bicarbonate – as shown by the fragment (g*-h*).

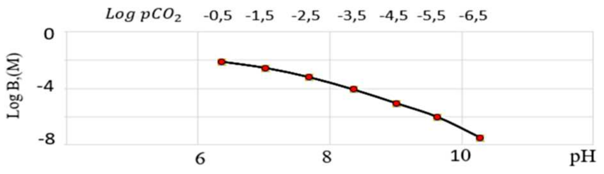

Figure 6. Effect of CO_2 partial pressure on the buffer capacity (B) of the aqueous medium in an open carbonate system.

The increase of pH in the process of blowing the solution of calcium chloride with sodium bicarbonate in the excess of the latter occurs due to the shift of the reaction of hydrolysis of bicarbonate ions to the accumulation of hydroxyl ions and carbonate ions, as (Equation (22)) shows. This is confirmed by the results of the pH measure in the initial calcium-free solution, the behavior of which when blown by a stream of air bubbles is shown by the black graph in sections (b-c) and (c-d). Fragments (b-c) and (c-d) are pH changes caused by hydrolysis of bicarbonate ions, volatilization of carbon dioxide from the water volume to the atmospheric air, and accumulation of hydroxyl and carbonate ions in the water. The steep pH drop in section (d-e) is the result of the binding of accumulated carbonate ions with calcium ions from the added calcium chloride. The final section (e-f) shows the pH change during the subsequent aeration-induced hydrolysis of added bicarbonate.

Figure 5B describes the meaning of the inflection points (e*) and (f*) on the pH vs. time graph (see red line in Figure 5A) in a solution of a mixture of calcium chloride and sodium bicarbonate, flushed with air and containing an excess of bicarbonate ions. At point (d*), the carbon dioxide begins to volatilize into the atmospheric air, facilitated by the flow of air bubbles. In this case, an excess of hydroxyl ions appears in the aqueous medium and, accordingly, an increase in pH, which is in accordance with (Equation (22)) for the case of an open calcium carbonate system. Then, for some time, the consumption of hydroxyl ions to produce carbonate ions according to (Equation (22)) and the binding of these ions to calcium ions to form insoluble calcium carbonate precipitate according to (Equation (24)) dominate. These reactions cause the pH to decrease. The rate of the processes is balanced by: the convection–diffusion transfer of carbon dioxide from the solution volume to the water–air interface, its decomposition into carbon dioxide and water at the interface surface, and the diffusion–convection transfer of carbon dioxide from the water–air interface into atmosphere.

Volatilization of carbon dioxide from the water in a calcium-containing carbonate system reduces its buffer capacity (B). This value corresponds to the amount of strong acid or alkali that will shift the pH by one unit. Figure 6 shows the effect of pH on the buffer capacity of an open carbonate system; above the pH scale in this figure is a scale of conjugate values of decimal logarithms of the partial pressure of carbon dioxide in the boundary air (ranging from carbon dioxide-saturated soil air to highly carbon dioxide-depleted atmospheric air).

As the carbonate system transfer from an initially open state to a closed state (deprived of access to atmospheric air), its buffer capacity remains approximately the same as its predecessor. When the initially closed carbonate system becomes open, its buffer capacity changes depending on the concentration of dissolved carbon dioxide and the concentration (partial pressure) of carbon dioxide in the atmospheric air. The activities of carbon dioxide in water and atmospheric air equalize over time. Correspondingly, the concentrations of dissolved carbonate components in water and the pH increase or decrease. The timing of the transition to a new equilibrium state depends on the fluxes of carbon dioxide from the aquatic environment to the atmosphere and back.

3. Conclusions

A denitrifying biofilter enriches the filtrate with bicarbonate and hydroxyl ions because of the activity of denitrifying and sulfate-reducing bacteria. Denitrifying bacteria are the main contributors to the saturation of water with these ions. Using ethanol as a substrate, denitrifying bacteria produce 0.87M bicarbonate ions and 0.13M hydroxyl ions for every mole of nitrate ions they remove from the water. Most of the resulting bicarbonate ions remain in the filtrate in their native form unless they have access to atmospheric air. Subsequent aeration of the filtrate results in processes that restore the dynamic equilibrium between the concentrations of all dissolved and undissolved carbonate components, the pH and the concentration of carbon dioxide in the aqueous medium relative to its content in atmospheric air. Facilitated hydrolysis results in a rapid decrease in the concentration of hydrogen ions and an increase in the concentration of carbonate ions in the water bulk of carbonate system. The addition of calcium ions to the aerated filtrate results in the binding of excess carbonate ions to form insoluble calcium carbonate. As a result, the pH in the filtrate decreases to the equilibrium value corresponding to the partial pressure of CO_2in atmospheric air. If tap water contaminated with sodium nitrate to a concentration of 250 mg/L (3.10-3 M) and with an initial hardness of 3.75 mg/eq/L is fed to biological denitrification, the nitrate concentration in the water leaving the biofilter become below 10 mg/L and the hardness reduced to 1.87 mg/eq/L. This is due to the binding of carbonate ions (i.e. hydrolysis products of hydro-carbonate ions) to calcium ions present in the water, as part of its permanent hardness. Calcium carbonate releases from the filtrate as a dispersed phase. The lack of ‘free’ calcium ions in the denitrified water in relation to the quantity of carbonate ions formed causes a partial reduction in the hardness of the filtrate and an increase in its carbonate alkalinity. During the denitrification of highly nitrate-polluted water with initially high constant calcium hardness, the total hardness index in the aerated filtrate decreases in proportion to the degree of conversion of nitrate ions into molecular nitrogen. Conclusions are based on the results of pH variation in the filtrate vs time in response to internal disturbances (biochemical reactions) and external influences (degassing conditions). The method of pH measurement with time sweep complements the data of pH measure in equilibrium states and allows one to reveal in more detail the peculiarities of the behavior of the carbonate system in the submerged denitrifying biofilter. The method has no fundamental limitations and makes it possible to monitor the rates of processes associated with changes in acid–base equilibria in various natural and model objects.

Authors contribution

Gevod V.S., Conceptualization, Investigation, Data curation, Writing – original draft.
Borysov I.A. Performing of experiments. Data curation, Formal analysis, Methodology and Writing.
Kovalenko I.L. Data curation, Editing, and supervision.
Frolova LA. An explanation of the context and outcomes of mathematical modeling.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

All data generated or analyzed during this study are included in this article.

References

[1] Taniguchi, M.; Wang, K.; Gamo, T. *Land and Marine Hydrogeology*; Elsevier Science, **2003**.

[2] Garrels, R.M.; Christ, C.L. *Solutions, Minerals, and Equilibria*; Harper and Row: New York, **1965**.

[3] Asmael, N.M.; Huneau, F., Garel, E., et al. Hydrochemistry to Delineate Groundwater Flow Conditions in the Mogher Al Mer Area (Damascus Basin, Southwestern Syria). *Environ Earth Sci* **2014,** *72*, 3205–3225. DOI:10.1007/s12665-014-3226-5.

[4] Wei, L.; Wu, Q., Zhang, J., et al. Composition and Dynamics of Bacterial Communities in a Full-Scale Mineral Water Treatment Plant. *Front. Microbiol.* **2019,** *10*, 1542. DOI:10.3389/fmicb.2019.01542.

[5] WHO *Guidelines for Drinking-Water Quality [Electronic Resource]: Incorporating 1st and 2nd Addenda, Vol.1, Recommendations*; WHO: Geneva, **2008**.

[6] Ratzke, C.; Gore, J. Modifying and Reacting to the Environmental pH Can Drive Bacterial Interactions. *PLoS Biol.* **2018,** *16* (3), e2004248. DOI:10.1371/journal.pbio.2004248.

[7] Liu, X.; Zarfel, G., Van der Weijden, R., et al. Density-dependent Microbial Calcium Carbonate Precipitation by Drinking Water Bacteria via Amino Acid Metabolism and Biosorption. *Water Res.* **2021,** *202*, 117444. DOI:10.1016/j.watres.2021.117444.

[8] Görgen, S.; Benzerara, K., Skouri-Pane, F., et al. The Diversity of Molecular Mechanisms of Carbonate Biomineralization by Bacteria. *Discover Materials* **2021,** *1*, 2. DOI:10.1007/s43939-020-00001-9.

[9] Wang, Y.; Wang, Z., Ali, A., et al. Microbial-induced Calcium Precipitation: Bibliometric Analysis, Reaction Mechanisms, Mineralization Types, and Perspectives. *Chemosphere* **2024,** *362*, 142762. DOI:10.1016/j.chemosphere.2024.142762.

[10] Liu, Y.; Ali, A.; Su, J.F.; Li, K.; Hu, R.Z.; Wang, Z. Microbial-induced Calcium Carbonate Precipitation: Influencing Factors, Nucleation Pathways, and Application in Waste Water Remediation. *Sci. Total Environ.* **2022,** *860*, 160439. DOI:10.1016/j.scitotenv.2022.160439.

[11] Hammes, F.; Boon, N.; Clement, G.; De Villiers, J.; Siciliano, S.D.; Verstraete, W. Molecular, Biochemical and Ecological Characterisation of a bio-Catalytic Calcification Reactor. *Appl Microbiol and Biotechnol* **2003,** *62* (2-3), 191–201. DOI:10.1007/s00253-003-1287-6.

[12] Gevod, V.S.; Chernova, A.S. Water Denitrification by Displacement Biofiltration. *Voprosy Himii I Himičeskoj Tehnologii* **2021,** *4*, 11–20.

[13] Gevod, V.S.; Chernova, A.S., Zhumadulayeva, A.I., et al. Buffer Effects in Submersed Denitrifying Biofilter. *J. Ecol. Eng.* **2022,** *23* (4), 145–154. DOI:10.12911/22998993/146634.

[14] Mohseni-Bandpi, A.; Elliott, D.J.; Zazouli, M.A. Biological Nitrate Removal Processes from Drinking Water Supply Review. *J Environ. Health Sci. Eng* **2013,** *11* (1), 11–35. DOI:10.1186/2052-336X-11-35.

[15] Gevod, V.; Borysov, I.; Frolova, L.; Issayeva, A. Influence of the Nutrition Substrate Concentration on Sulfate Reduction in Denitrifying Biofilter Point of use. *Indian Chem. Eng.* **2023,** *65* (5), 439–450. DOI:10.1080/00194506.2023.2205851.

[16] Pankow, J.F. *Aquatic Chemistry Concepts*; CRC Press: Boca Raton, **2019**. DOI:10.1201/9780429198861

[17] Smith, K.F.; Oram, D.M. Corynebacteria (including diphtheria). In *Encyclopedia of Microbiology*, 3rd ed.: Schaechter, M., Ed.; Elsevier, **2009**; pp. 94–106.

[18] Hammad, I.A.; Talkhan, F.N.; Zoheir, A.E. Urease Activity and Induction of Calcium Carbonate Precipitation by Sporosarcina Pasteurii NCIMB 8841. *J. Appl. Sci. Res.* **2013,** *9* (3), 1525–1533.

[19] Pérez, H.F.; García, M.G. Bioprecipitation of Calcium Carbonate by Bacillus Subtilis and its Potential to Self-Healing in Cement-Based Materials. *Journal of Applied Research and Technology* **2020,** *18* (5), 245–258. DOI:10.22201/icat.24486736e.2020.18.5.1280.

[20] Mitchell, A.C.; Espinosa-Ortiz, E.J., Parks, S.L., et al. Kinetics of Calcite Precipitation by Ureolytic Bacteria Under Aerobic and Anaerobic Conditions. *Biogeosciences.* **2019,** *16* (10), 2147–2161. DOI:10.5194/bg-16-2147-2019.

[21] Wang, L.K.; Shammas, N.K. Single-Sludge Biological Systems for Nutrient Removal. *W: Advanced Biological Treatment Processes, Series: Handbook of Environmental Engineering* **2009,** *9*, 209–270. DOI:10.1007/978-1-60327-170-7_7.

[22] Bomberg, M.; Mäkinen, J.; Salo, M.; Arnold, M. Microbial Community Structure and Functions in Ethanol-Fed Sulfate Removal Bioreactors for Treatment of Mine Water. *Microorganisms.* **2017,** *5* (3), 61. DOI:10.3390/microorganisms5030061.