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USE OF AUTO-INDUCED SURFACTANTS FOR CLARIFICATION OF BIODENITRIFIED WATER BY BUBBLE-FILM EXTRACTION METHOD

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Abstract. The process of getting clarified denitrified water in a biofilter used combined methods of displacement (piston) biofiltration and bubble-film extraction is studied. It is shown the products of bacterial metabolism released into the water at biofiltration have surfactant properties. They can serve as collectors of the dispersed phase to achieve the desired degree of clarification of water when using bubble-film extraction. The turbidity of the resulting denitrified water does not exceed sanitary and hygienic limits. The concentration of biosurfactants is also significantly reduced.

Keywords: denitrification, displacement biofiltration, bubble-film extraction, clarification.

1. Introduction

The high concentration of nitrates in groundwater (≥45 mg/L) has become one of the key environmental issues of the last decades. This is due to the negative implications of this chemical on human and animal health. The use of nitrogen fertilizers in crop production, discharge of insufficiently treated wastewater from livestock and poultry farms, as well as industrial effluents have increased the nitrogen load into receiving waterways.[1] Nitrates enter the human body through the consumption of nitrate-contaminated groundwater for cooking and drinking. Excessive concentration of nitrates in the water cause many health disorders in humans, namely, methemoglobinemia, gastric cancer, goiter, birth malformations, hypertension, *etc.*[2] Various nitrogen removal processes are used at drinking water treatment plants (DWT). They are the ion exchange filtration, adsorption filtration, electrochemical reduction of nitrates, and electro-dialysis, reverse osmosis, and microbiological reduction of nitrates to molecular nitrogen in fluidized bed reactors and biofilters.[3] Microbiological denitrification seems now the most economical and environmentally friendly method of nitrate removal from contaminated water. This method is reasonable to use not only at the centralized water treatment plants. A good result one can get is also applying it in decentralized water treatment systems, as well as in the equipment of point of use.[4,5]

When one selects to realize the biological denitrification in small size devices, fixed carriers of a certain size are reasonable to be used for attaching bacterial cells and growth of their colonies (incubation of denitrifying biofouling). The use of support particles with a certain size and ratio of their cross-section to the surface area allows the reaction of microbiological denitrification to run with the required rate and without clogging the filtration bed.

The bacteria able to denitrify nitrate contaminated water are *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Gallionella*, *Halobacterium*, *Halomona*, *Hyphomicrobium*, *Janthinobacterium*, *Neisseria*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Rhizobium*, *Rhodobacter*, *Thiobacillus*, *Thiosphaera*, *Vibrio*, and *Xanthomonas*.[6,7] The enzymes associated with denitrifying bacteria are synthesized when outer conditions become advantageous. The synthesis of denitrifying enzymes is a specifically regulated process and typically occurs under anaerobic conditions. But in some cases, enzyme induction runs at a certain quantity of dissolved oxygen. An important requirement for bacterial reduction of nitrates to nitrogen gas is the presence in the water of a carbon source as the electron donor. Various solid, liquid and gaseous carbon sources are known as that. In current literature using of ethanol, methanol acetic acid, fatty acids, *etc.*, was described.[6] The efficiency of the process is very high and can reached about 100 %, which is not matched by any other methods available for nitrate reduction. However, the potential bacterial contamination of treated water and increasing its turbidity by bacterial metabolic products and byproducts is the main disadvantage. This risk is legitimate and the subsequent treatment (clarification)[8,9] and disinfection of water need to satisfy tap water standards.

This work was aimed at concurrent reduction of nitrates to nitrogen gas and removal of particulate matter (planktonic particles) of denitrification bacteria metabolic

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products and byproducts by applying conjugated biofiltration and advanced flotation (the bubble-films extraction). It was shown that the auto-induced bio-surfactants act as collectors of particulates and colloid species at the process of the Bubble-Films Extraction. The auto-induced biosurfactants are naturally released metabolic products of bacterial cells. They are entering the water and accumulate there from the biofoulings of filtration bed.

2. Experimental

2.1 Theoretical Background

The approaches to bioremediation of water contaminated with nitrates are generally similar when realized in large and small-scale DWT plants. In big DWT supplied tap water into large networks for a lot of people, the technologies of nitrates removal include the stages of anoxic treatment, aeration, settling, and final filtration. The last process requires the use of granular filtration beds (sand, charcoal, zeolite, *etc.*,[10] with their regular back-washing), or special filtering units – membranes of desired permeability. They also need maintenance. Less often the flotation technique is applied. It is because regular flotation requires the expenditure of special chemicals (surfactants) acting to collect admixtures of water on the surface of the up-floating air bubbles. The flow of air bubbles with captured admixtures, when reaching the water surface, is being removed from it as the foamy layer of flotation waste. The chemicals used as collectors of particulates of water turbidity typically are products of synthetic organic chemistry and is characterized with an essential carbon footprint. Most of them are slowly degradable and this is an important argument for not using them.

Last time within the philosophy of blue technologies and green chemistry a great interest is devoted to using in technical applications the collectors produced at the vital activity of microorganisms. Collectors of microbiological origin – biosurfactants are surface-active substances that are excreted by a wide range of microbes including the bacteria of denitrifying biofouling. Biosurfactants are divided into two major groups: low molecular mass which reduces surface and interfacial tension, and high molecular mass polymers those exhibit foam-forming and emulsifying properties. These substances have several advantages over the chemical surfactants such as higher biodegradability, lower toxicity, better environmental compatibility, high selectivity, foaming efficiency, and specific activity under extreme conditions such as temperature, pH, and salinity.

The interest in biosurfactants action in tap water first arose in the last quarter of the twentieth century. At that time one of the main reasons for worsening the quality of tap water in extended water networks was discovered. It was stated the surface-active substances of microbiological origin (biosurfactants) are released by biofoulings grown on the inner walls of water pipes and stipulate an increase of the water turbidity and its foam-forming. These substances accumulate in quantities of order a milligram per liter and more at the time of the water transporting through pipelines to a long-distance. They consist of soluble decay products of bacterium cells, non-soluble particulates of exopolysaccharides, and amphiphilic matter released by alive bacterium cells. The degree of saturation water by these substances essentially increases in the summertime of the year. Bacterial surfactants are capable of spontaneous assembling at the air-water or water-oil interface and thereby reducing the surface (interfacial) tension due to their hydrophilic and hydrophobic constituent parts.

Based on chemical structure, biosurfactants are divided into five major classes. They are lipopeptides, glycolipids, phospholipids, neutral lipids, and polymeric compounds.[11] Many biosurfactant-producing microorganisms are isolated and identified as producers of amino acids-containing biosurfactants. Other are identified as producers of glycolipids. *Thiobacillus*, *Aspergillus*, *Candida*, *Corynebacterium*, *Micrococcus*, and *Acinetobacter* are identified as producers of phospholipids and fatty acids. Biosurfactants with high interfacial and biological activity – lipopeptides are mainly produced by *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus*, *Serratia*, and *Actinoplanes*.[12]

Currently, about 90 lipopeptide compounds in 26 families have been identified. The majority of them belong to the cyclic compounds (86 compounds in 24 families).[13]

Extracellular biopolymers as the constituent part of the planktonic matter in the bio denitrified water are vast and grouped into classes: polysaccharides, polyesters, and polyamides. Their functions include adherence of cells to surfaces, migration, protection from drying, etc.

Polysaccharides are the most abundant, and their location relative to the cell forms the basis for consequent classification. At the cell wall, polysaccharides perform structural and protective functions. Outside the cell, they may take the form of a covalently bound cohesive layer (a morphologic entity termed capsule) or completely excreted into the environment as slime. The capsules serve as adherents of cells to surfaces and may be overproduced when there is an abundance of sugars to become reserves of carbohydrate for subsequent metabolism.[14] Dextran is a good example of this group. The distinction between loosely attached and unattached extracellular polymeric

substance lies in the structural and functional relationship with the cell. The appearance of these substances in the water bulk leads to an increase in its turbidity.

Most of the matrix exopolysaccharides are long-chain species with a molecular weight of 500-2000 kDa. They can be homo-polymers such as cellulose, curdlan, dextran, or hetero-polymers like alginate, emulsan, xanthan. Exopolysaccharide chains can be linear or branched and are generally constituted by monosaccharides and some non-carbohydrate substituents such as acetate, pyruvate, succinate, and phosphate. Polysaccharides can interact with themselves and with ions, lipids, and proteins. These interactions result from electrostatic attractive and repulsive forces, the formation of hydrogen bonds and the action van der Waals forces. All listed forces influence the structure and the stability of the biofilm matrix and its complexes with other substances. Which kind of forces are dominative, depends on the chemical structure and spatial organization of interacting objects, as well as on the pH, ionic strength, and the salt structure of the aquatic environment. The water when flowing through the filtration bed inside of the dentrifying device captures fragments of biological matter from biofouling and thereby increases own turbidity.

To decrease the concentration of planktonic particulates in the denitrified water the bubble-films extraction method is suitable. The principle of this method consists of the adsorption of the surface-active admixtures of water on the surface of up-floating air bubbles and of following transforming of the arisen flotation product into the flow of thin liquid films (flotation waste). This flow is easily removable out of treating water through the channel of a certain geometry (bubble-films extractor). The peculiarities of the practical application of this method for the afterpurification of secondary contaminated tap water described in our previous work.[15] The unique advantage of the bubble-films extraction is its ability to withdraw surface-active admixtures and their byproducts from contaminated water at concentrations below 1 mg per liter. At that concentration surface-active active substances do not form a foamy layer at the water mirror and their removal by the method of regular bubbling flotation is problematic.

2.2. Materials and Methods

Denitrification of contaminated water was done by the method of displacement bio-filtration (reduction of nitrates to the Nitrogen gas) in the U-shaped filtering device.[4] This device was assembled from two pieces of PVC pipes with the inner diameter of 100 mm, the height of 1500 mm, a blanked bottom, and a hydraulic jumper with an outlet valve disposed at the height of 50 mm from the blanked bottom. On the upper part of each of the pipes forming a U-shaped construction, the holes of radius 10 mm were made at the distance of 150 mm and 300 mm from their open ends, and suitable fittings with water taps were mounted to supply water onto filtration and for the drain of filtered water. In such a way, two functional compartments with the height of 150 mm were made for extra filling with water each elbow of U-shaped biofilter. This allowed the installation of the bubble-films extractor into the output bend of the biofilter, and to study in more detail the dynamics of the denitrification process in the water filling the entering part of the device. Each of the functional sections (compartments) contained 1.25 L of water in addition to water volume which fills the filtration bed itself. In the inlet elbow of the bio-filter, water was moved in a top-down direction, and in the outlet elbow, from bottom-up. It was also possible to take water samples for analysis from the bottom part of the bio-filter. To carry out denitrification the HDPE filter media consisting of profiled hollow rollers with denitrifying biofouling grown on their surface was used. The caliber of the bed rollers was 16 x 12 mm, the surface area in bulk -1000 m^2/m^3, the specific volume of free space - 0.75 m^3 per 1 m^3 of bulk material. In each of the bio-filter elbows, the filtration path length was equal to 1200 mm. The volume of water filling of the biofiltration bed was 15 dm^3, and the overall volume of water in the operating device – 17.5 dm^3. Sodium nitrate solutions in tap water were used for biofiltration. The concentration of the solutions was 100–750 mg/L. Ethanol was added to each of them in a 1.5-fold excess as compared to the stoichiometrically necessary for complete bio-denitrification.

Before being an object in this study, the biofilter of the described design was in operation for about a year for everyday denitrification in the displacement mode of 2.5 L of tap water which was polluted by nitrates in the range of concentrations of 100–600 mg/L with adding of ethyl alcohol in required quantities as a source of easily consumable carbon.

The bubble-films extractor used in this study was of the following parameters. The capturing funnel and evacuating channel were of dimensions: diameter and height of funnel – 60 × 60 mm, inner diameter, and height of evacuation channel – 18 × 180 mm. The distance of the air disperser (air stone) apart the base of the capturing funnel was equal to 60 mm, air discharge – 1 dm^3/min, and the radius of generated air bubbles by air stone – 0.2–1.5 mm.

The surface activity of biosurfactants secreted from biofouling into the filtered water was measured by the Wilhelmy method. A platinum plate with the dimensions 50 × 10 x 0.1 mm was used in conjunction with the analytical ultra-weights Sartorius. The sensitivity of instrument – 0.01 mN/m. Surface tension was measured in

the selected samples of water, filled into the Petri dish (samples volume – 50 mL, surface area – 100 cm^2).

The nitrate concentration was measured by I-160 MI ionomer using an ELIS-121NO_3 ion-selective electrode. The range of measure of the concentration of nitrate ions was $1 \cdot 10^{-1}$ – $5 \cdot 10^{-5}$ M. The sensitivity – $5 \cdot 10^{-6}$ M. Turbidity of the water samples was measured by Portable turbidity Meter MA-267.

At mathematical processing and graphical display of the obtained results, the actual data of the measured values were calculated as the arithmetic average of three consecutive measurements. The correspondence between the model kinetic equations and the experimental data was checked by calculating the standard deviation between data arrays using Excel 2016 software.

3. Results and Discussion

The biofilter of the described design allows one the everyday receiving of a certain quantity of denitrified water when an equal quantity of water, contaminated by nitrates is supplied synchronously to the apparatus in one gulp. In this study, the portions of the supplied and received water were equal to 2.5 L. The peculiarities of biofiltration illustrate the graphs in Fig. 1.

The upper left part in this figure shows the dynamics of the process when a biofilter with the denitrifying biofouling in its pore space has been completely freed from water through the bottom water tap in the hydraulic jumper and filled by water again with a certain concentration of NO_3^-. In this case, the stagnant water state is inside the biofilter. At this mode, the concentration of nitrates *vs.* time reduces as depicted in curves 1–4 for the range of time 4–15 days. If after the run at the water stagnant state regime, the biofilter is switched to operate in the displacement mode with every day feeding the discrete portions of the water of certain nitrates concentration to its entrance and synchronous receiving of denitrified water at its exit, then denitrification occurs as depicted beyond the mark of 15 on the time ax. At different initial concentrations of nitrates in portions of supplied water (curves 1-4), the concentration of nitrates in the water at the output of the biofilter does not exceeds few mg/L. It is shown by the bottom curve at the right-hand graphs in Fig. 1 (time interval 15–23 days). As seen, the influence of initial nitrates concentration in entering the water on the concentration of nitrates in the portions of processed water is very soft.

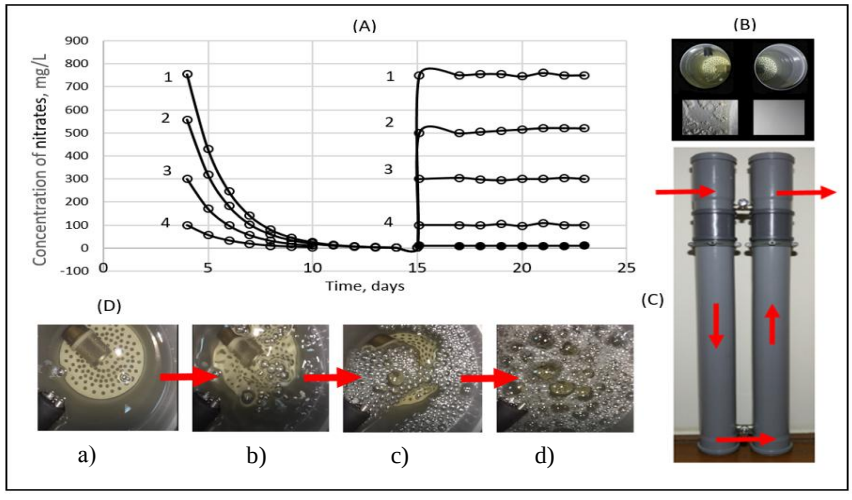

Fig. 1. (A) – The dynamics of bio-denitrification of nitrate contaminated water at different modes of the U-shaped biofilter functioning. (B) – The photos of open-top sections of the biofilter and the photos of biofouling at the entering and exit elbows of this device. (C) – The photo of the U-shaped biofilter and the trajectory of the water flow in it. (D) – Photos of the foam appearance at the time-course of mechanical vibration of the device elbows

At the biofilter functioning, the nitrogen gas, particulate matter (planktonic particles) of denitrifying biofouling and soluble biosurfactants release to the filtrate. The nitrogen forms the gas bubbles inside the biofiltration bed. After the growth to a certain size on the surface of biofouling, the bubbles under the action of the Archimed force move up through the large pores in the filtration bed and finally are transferred to the atmosphere. The equations of bio-denitrification at bacterial respiration and bacterial synthesis are the followings:

$$12NO_3^- + 5C_2H_5OH \rightarrow 6N_2 + 10CO_2 + 9H_2O + \\ + 12OH^- \quad (1)$$

$$97NO_3^- + 55C_2H_5OH + 15O_2 \rightarrow 5C_5H_7O_2N + \\ +85CO_2 + 99\,H_2O + 46N_2 + 97\,OH^- \quad (2)$$

Eq. (2) differs from the one published in earlier.[16] The difference is the additive 15 O_2 on the left-hand side. This additive describes the requirement of heterotrophic denitrifying bacteria some free oxygen to synthesize important adaptive enzymes in their cells.

Taking the sum of Eqs. (1) and (2), one gets:

$$109NO_3^- + 60C_2H_5OH + 15O_2 \rightarrow \\ \rightarrow 5C_5H_7O_2N + 95HCO_3^- + 108H_2O + 24OH^- + \\ +52N_2 \quad (3)$$

or:

$$NO_3^- + 0{,}55C_2H_5OH + 0.137O_2 \rightarrow \\ \rightarrow 0{,}046C_5H_7O_2N + 0{,}87HCO_3^- + \\ +0{,}99H_2O + 0{,}13OH^- + 0{,}48N_2 \quad (4)$$

From Eq. (4), it follows each gram-mol of nitrates transforms inside the biofilter into approximately half of the mole of the nitrogen gas. This process is accompanied by the consumption of 0.55 mol of ethyl alcohol and 0.137 mol of dissolved oxygen. The course of reaction releases 0.99 mol of H_2O and 0.13 mol of hydroxide ions. Processing of water with the nitrate concentration of about 500 mg/L leads to the volume of released nitrogen gas to 0.25 L/ day. The gas evolution through the filtration bed occurs in the form of up-floating bubbles. The bubbles appear and grow due to the functioning of denitrifying bacteria. The bubbles visually manifest themselves rising through the water located above each filtration bed in both elbows of the U-shaped device. The mechanical activation (vibrating) of the devises elbows with a frequency of about a few Hertz and the amplitude of order 1 mm, leads to the rapid releasing of Nitrogen gas bubbles out of the filtration bed. The process is accompanied by the arising of the foamy layer at the surface of the water in both elbows. The intensity of the process is much higher in the entering elbow. The typical picture is shown by photos "a,b,c,d" in Fig. 1D. Under mechanical stress, the time of the nitrogen bubbles releasing takes about one minute. The generated foam disappears rather quickly, also.

The foam appearing indicates to delivery and release of biosurfactants onto the air-water interface by the bubbles of nitrogen gas. The surfactants releases from the surface of nitrogen bubbles being burst at the surface of the water when they are transferred to the atmosphere.

Visual observations showed also that at the chronicle operation of the biofilter, powerful biofouling appeared on the inner surface of the entering elbow. Water samples taken off this elbow are less transparent and of yellowish hue. In the output elbow, biofouling does not manifest itself to such an extent, and denitrified water is visually transparent and colorless. The photographs of the input and output compartments of the biofilter taken from above through the open ends of the device are shown in Fig. 1B. Here the photographs of the internal wet surfaces of the input and output compartments, taken immediately after the drainage of water are also represented. This indicates that soluble biosurfactants and nonsoluble planktonic particulates are released from denitrification biofilms (biofouling) and accumulates in the water during bio-denitrification.

The biosurfactants are lipopeptides, glycolipids, phospholipids, neutral lipids, and polymeric compounds. All of them possess hydrophobic moieties and hydrophilic parts. The size of the hydrophobic moieties ensures amphiphilic properties for these substances. The structure of hydrophilic groups makes it possible for biosurfactants species to interact with other substances through the formation of chemical bonds, electrostatic links, and hydrogen bonds. The hydrophobic moiety is a key factor that allows dissolved biosurfactants to accumulate at the surface of the water.

To evaluate the surface activity of biosurfactants released denitrifying biofoulings, the samples of water were taken regularly from the entering and exit compartments in the functioning device, and their surface tension *vs.* time was measured by the Wilhelmy method. That data was compared with the surface tension of the initial water contaminated by nitrate and with the surface tension of the flotation concentrate produced by the bubble-films extractor at the treatment of denitrified water. The results are shown in Fig. 2 for the case of the study denitrification process when processed the water with an initial concentration of $NaNO_3$ equal 500 mg/L.

The left upper part in Fig. 2 shows the results of measuring of the surface tension of the initial water (contaminated by nitrate), as well as the water at the exit of the biofilter before and after its treatment by the bubble-films extractor (curve with the indexes 1,3,5). The results of the study of the water sample taken from the entering compartment in the biofilter (curve 2) after 24 hours of starting the displacement biofiltration process are also shown (that is, 24 hours after filling the device with another portion of water intended for processing). On the right-hand side of Fig. 2, the data of the measurement turbidity in the studied samples are represented.

The samples of supplied water (1), denitrified water (3) as well as denitrified water after their processing with the bubble-films extractor (5) exhibit no change in the surface tension vs time. That is the surface tension remains practically constant at the course of the experiment and is equal to 73.5 mN/m. These results indicate the minor concentration of biosurfactants in the studied samples and minor adsorption of these surface-active substances at the air-water interface when studied the initial water (supplied to biofilter) and of denitrified water (obtained at the exit of biofilter).

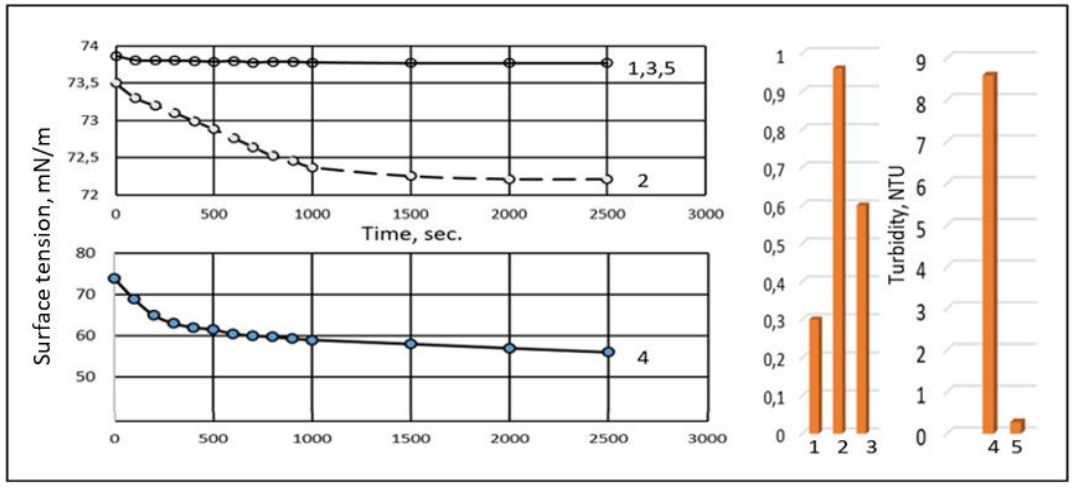

Fig. 2. The results of the measure surface tension of water samples *vs.* time which have been taken at the course of bio-denitrification and the results of measure turbidity of that samples. Explanations in the text

However, although incoming water does not show any change of surface tension *vs.* time, the probes of water that been regularly sampled from the entering compartment of the biofilter 24 hours after its filling with each previous portion of water intended for processing exhibit the surface tension decrease *vs.* time from 73.5 to 72.2 mN/m as shown by curve 2. This indicates marked delivery and adsorption of biosurfactants on the surface of the water with up-floating bubbles of nitrogen gas released at the process of bio-denitrification.

The left bottom part in Fig. 2 (curve 4) represents the data of measurement surface tension *vs.* time in flotation concentrate when it's obtained at the processing of filtrate by the bubble-films extractor. This result indicates despite the low concentration of biosurfactants in the denitrified water the bubble-films extractor ensures effective removal of these substances out of the biofiltrate. As a result, the flotation concentrate shows the behavior of a typical surfactant solution of moderate concentration. Its surface tension decreases over time from the initial level of 74 mN/m to the final level of 55 mN/m. This result coincides with the data published elsewhwere.[12]

The right-side plots in Fig. 2 show the results of turbidity measurements in the water samples. Column 1 refers to the sample of initial water. Column 2 – the sample of the water has been taken at the exit of the biofilter, and column 3 – the sample of water has been taken from entering compartment of the biofilter after 24 hours running of displacement biofiltration. For comparison shown the turbidity of flotation concentrate releases from the bubble-films extractor at the processing of denitrified water (long column 4) and the turbidity of denitrified water after its processing by the bubble films extractor (very short column 5). The turbidity of flotation concentrate is 5-10 NTU units, the turbidity of none clarified bio filtrate – 0.56-0.8 NTU units, and the turbidity of the bio-filtrate after its clarification by the method of bubble-films extraction – 0.16–0.28 NTU.

In such a way, the bubble-films extractor withdraws biosurfactants and planktonic particles that appear in the water. As a result, the turbidity in the filtrate becomes below of sanitary-hygienic limit.

The scheme of the biofouling and detachment planktonic matter into the stream of been filtering water is shown in Fig. 3. This picture in the main is like described elsewhwere.[14]

Biosurfactants is been at low concentration in water adsorb at the water surface in quantities described by Eq. (5):

$$\Delta\gamma \frac{1}{G} = RT \tag{5}$$

where $\Delta\gamma$ is the magnitude of reducing the surface tension; G is adsorption; R is the universal gas constant; T is absolute temperature.

The values of $\Delta\gamma$ corresponding to differences of the initial and final states of the surface tension displayed by curves 2 and 4 in Fig. 2, inserted into (5) give the values of adsorption G equal $5.48 \cdot 10^{-7}$ mol/m^2 and G equal $8 \cdot 10^{-6}$ mol/m^2 respectively. These results indicate the arising of the gaseous adsorption monolayer in the first case and the condensed monolayer in the second case.

Here is required to note despite low dynamic adsorption of biosurfactants on the surface of up-floating air bubbles, nevertheless, the bubble-films extractor evacuates effectively these substances from the biofiltrate.

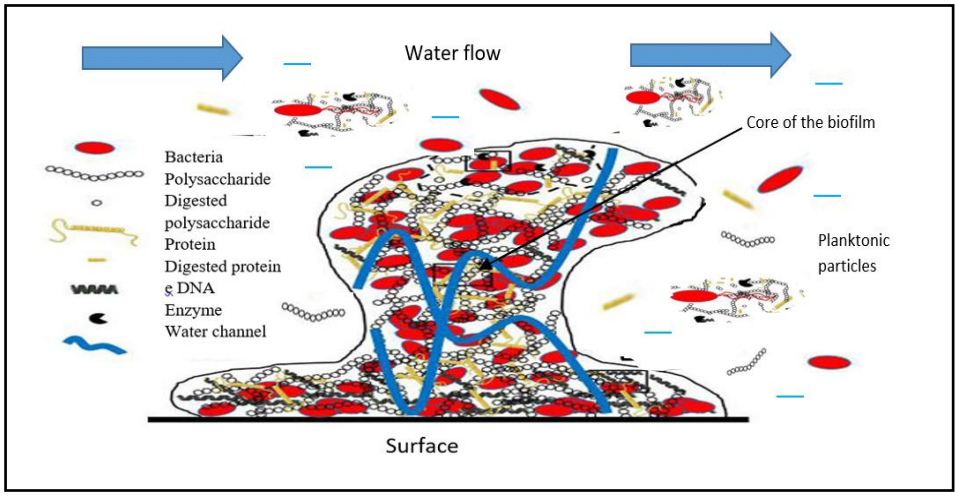

Fig. 3. Schematic representation of a mature biofilm and its constituents. In the center, the overall picture of the structure of the biofilm: bacteria are attached to the solid surface and included in a self-induced polymer matrix. All exocellular compounds form a protective gel around the microorganisms. In the biofilm detachment area, microbial enzymes destroy the exopolymeric matrix and release the cells that regain mobility, to be able to colonize new surfaces. Other components of the biofilm also partially pass into the water. Biosurfactants are among them

The equation describing the process of surfactants removal by the bubble-films extraction[15] is as follows:

$$\frac{C}{C_0} = exp\ \ -\frac{K\tau}{V} \qquad (6)$$

where C is final concentration of biosurfactant in the processed water; C_0 is initial concentration of bio-surfactant in the water entered to processing; τ is current time; V is the volume of treating water; and K is the rate constant of the bubble-films extraction. The value of K describes Eq. (7):

$$K = \frac{3W_{air}\,h}{K_{eq}\,r}\quad 1 - exp\ -\ K\uparrow + K\downarrow\ \frac{l}{\underline{V}} \qquad (7)$$

where W_{air} is the air discharge for bubbling; K_{eq} is the constant of equilibrium adsorption; r is the radius of air bubbles; h is the thickness of adsorption layer; $K\uparrow$ is the rate constant of surfactant adsorption; $K\downarrow$ is the rate constant of surfactant desorption; $l\ \underline{V}$ is the lifetime of air bubbles in the bulk of water (from the moment of their appearance at the depth ℓ and ascents at a speed $\underline{V}$ before reaching of water mirror).

Planktonic particulates that contaminate filtrate at the course of biological denitrification are the detached fragments of biofouling. They are released into the water as individual extracellular biopolymeric matter as well as the aggregates with incorporated bacteria. These particles interact with proteins, polypeptides, lipopeptides, glyco-lipids, phospholipids, neutral lipids, and ions. In the work,[17] the charges of biofoulings *vs.* pH have been studied. An excess of negative charges in an alkalic medium and positive charges in an acidic one was shown. So, in principle, the biosurfactant molecules interact with each other as well as with the exopolysaccharides *via* the formation of homo and hetero-associates. This is ensured by the formation of saline bonds (ionic interaction), hydrogen bonds, and associates linked by Van der Waals forces.

Thus, the nature itself has given the opportunity of flotation removal of the components of turbidity from the bio filtrate by the method of the bubble-films extraction. It is ensured by employing biosurfactants released by alive denitrifying biofouling. To confirm this turbidity variation inside processed water along the filtration pathway in the U-shaped biofilter with "on" and "off" the bubble-films extractor installed at the exit of this device was studied. The results obtained are shown in Fig. 4.

Two sets of experiments were carried out. The first of them was performed with the initial concentration of nitrates in the supplied water equal to 500 mg/L and the second one – 100 mg/L, using displacement (piston) mode of biofiltration. Two and a half liters of water have been filled to the processing into biofilter daily and equal portions of denitrified water were obtained synchronously. The turbidity in water along the pathway of biofiltration was checked the next day after the injection in one gulp of the portion of water to its processing. The mark (1) on the absciss ax at the right-hand in Fig. 4 corresponds to the initial turbidity of the water supplied to biofiltration, the mark (2) corresponds to the turbidity of the water in the entering elbow of the apparatus after 24 hours. The marks (3) and (4) correspond to the turbidity of water measured at the same time at the midpoint and the exit of the biofilter. The solid curve with the index (a') shows the

turbidity change in the water along the filtration path without using the bubble-films extractor and the curve with the index (b') indicates the turbidity of water along the filtration path with the use of the bubble-films extractor. The dashed curves show the turbidity of water along the filtration path after mechanical vibration to the biofilters elbows with the frequency of few Hertz and amplitude 1 mm for 15 seconds.

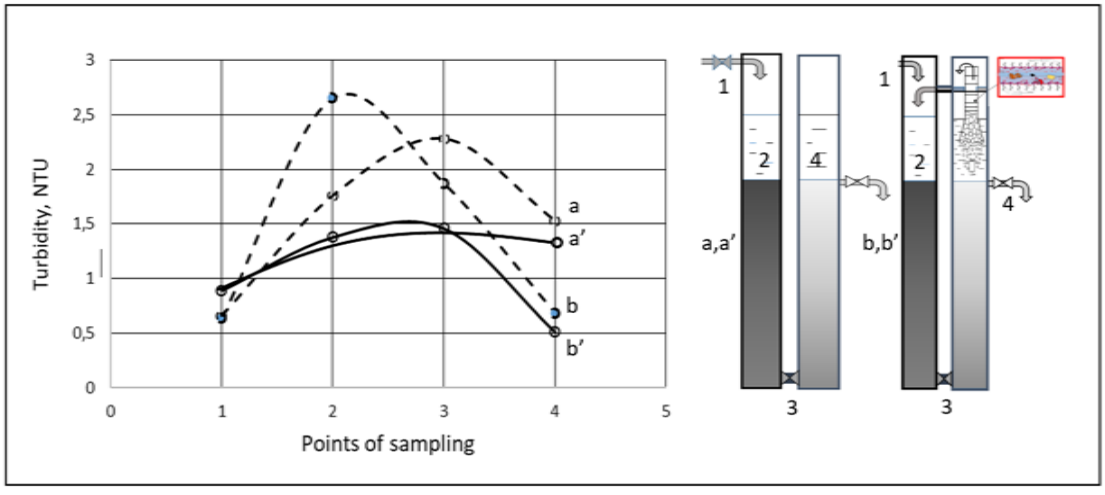

Fig. 4. The turbidity variation along the filtration pathway in the functioning U-shaped biofilter without and with bubble-films extractor at its exit (left part of the figure). The schematic of the apparatus with the depicting of sampling sites shown in the right part of the figure. Other explanations are in the text

As seen, in both cases the application of the bubble-films extractor leads to decreasing of turbidity in the received filtrate to the values below of sanitary-hygienic limit. Additionally, one can conclude the turbidity-make matter, when released by the denitrifying biofouling at the initial steps of the biofiltration pathway, becomes partially consumed by that at the next steps of the biofiltration. This conclusion follows from the bell-kind shape of solid and dashed curves with the indexes (a) and (a'), represented in the graphs, *i.e.*, the partial clarification of the bio filtrate occurs naturally at the course of its shifting along the filtration pathway. The application of bubble-films extractor at the exit of biofilter ensure the additional decrease of the bio filtrate turbidity in such a way that this indicator becomes below of sanitary-hygienic limit in any case.

4. Conclusions

The turbidity of the bio-denitrified water at the exit of manufactured U-shaped small size biofilter is possible to eliminate by the ecologically friendly method – the bubble-films extraction. The use of this method does not require expenditures on special synthetic surface-active substances for promotion accumulating (adsorbing) the turbidity particles on the surface of air bubbles applying flotation. The desired transparency of the filtrate can be attained using the collecting properties of naturally generated surfactants released by bacterium cells inside of the biofilter. These substances are metabolic products of denitrifying biofilm and naturally appear in the water in the course of biological filtration. When reaching a certain concentration in the bio filtrate these substances allow effective removal of turbidity particulates out of the denitrified water employing the bubble-films extractor. The turbidity of thereby processed bio denitrified water does not exceed a sanitary-hygienic limit. The concentration of biosurfactants is also reduced by many times. The system is simple, very reliable, highly performant, and of low operational cost.

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ВИКОРИСТАННЯ АВТОІНДУКОВАНИХ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН ДЛЯ ОСВІТЛЕННЯ БІОДЕНІТРИФІКОВАНОЇ ВОДИ МЕТОДОМ БУЛЬБАШКОВО-ПЛІВКОВОЇ ЕКСТРАКЦІЇ

Анотація*. Досліджено процес отримання освітленої денітрифікованої води в біофільтрі із застосуванням поєднання методів витіснювальної (поршневої) біофільтрації та бульбашково-плівкової екстракції. Показано, що продукти бактеріального метаболізму, що виділяються у воду в процесі біофільтрації, мають поверхнево-активні властивості і можуть виконувати функцію збирачів дисперсної фази для досягнення бажаного ступеня освітлення води при використанні бульбашково-плівкової екстракції. Каламутність (освітлення) очищуваної денітрифікованої води не перевищує санітарно-гігієнічних меж. Концентрація біосурфактантів також значно знижується.*

Ключові слова*: Денітрифікація, витіснювальна біофільтрація, бульбашково-плівкова екстракція, усунення каламутності.*