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WATER TREATMENT AND DEMINERALIZATION TECHNOLOGY

Seasonal Dynamics of Bacteria in Corrosive Biofilms Formed on the Surface of Wastewater Treatment Plants

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Abstract—The corrosive biofilm is formed on metal surfaces of wastewater treatment plants. The numbers of sulfate-reducing and ammonifying bacteria (up to 10^9 cell/cm^3 and up to 10^8 cell/cm^3, respectively) are dominant in this film, while the quantity of acidophobic tionic bacteria is the smallest (up to 10^2 cell/cm^3). The relationship between the bacteria-produced hydrogen sulfide and the measure of wastewater acidity has been established indicating that with the rise of hydrogen sulfide production the water pH decreases. The rate of steel corrosion under exposure to enrichment cultures of sulfate-reducing bacteria separated from biofilms depends on the season, separation of bacteria and their metabolic activity. The correlation between the number of iron-reducing bacteria, concentration of phosphate ions in wastewater and the rate of steel corrosion has been established.

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Keywords: biofilm, wastewater treatment plants, corrosive microbial community, seasonal dynamics.

INTRODUCTION

Wastewater treatment facilities are an important part of city infrastructure, while their stable and effective operation is the guarantee of environmental safety of hydrosphere. The peculiarity of operation of wastewater treatment plants is the biologically active medium stipulating the accretion of metal and concrete surfaces [1]. Corrosion processes in the formed biofilm proceed depending on the metabolic and corrosive activity of sulfidogenic microbial communities, and the rate of these processes according to the available data [2, 3] can reach up to 40 mm/year. The microbial corrosion is considered to be one of the main causes of emergency situations [4], in addition, the losses of constructional functions of individual parts of facilities can lead to unpredictable consequences and, consequently, to significant economic and environmental damage [5].

The main components of corrosive microbial community of biofilm formed and functioning on the metal surface consist of sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB), denitrifying bacteria (DNB), ammonifying bacteria (AB), acidophobic tionic bacteria (TB), and also the fermentive type bacteria (nitrogen-fixing bacteria (NiB)) of *Clostridium* genus capable of nitrogen fixation [6]. Functioning of biofilm determines the constant dynamic of the number of microorganisms, at the same time the spike of the number of sulfate-reducing bacteria up to 10^4–10^6 cell/cm^2 can lead to the transition of sulfidogenic microbial community of biofilm to corrosive (corrosion-active) state [7]. Therefore, the investigation of factors affecting the metabolic and corrosion activity of microbial community functioning in biofilm is an urgent problem. It should be noted that the microbial corrosion of concrete as the main construction material of wastewater treatment plants was thoroughly studied [3, 8, 9], while the issues of metal equipment corrosion failed to receive sufficient attention [10–12].

The purpose of this paper is to study seasonal dynamics of the number of main components of corrosive microbial community of biofilm formed on surfaces of metal structures of wastewater treatment works in the town of Chernihiv (Ukraine) and to determine the metabolic and corrosion activity of the specified microbial communities.

EXPERIMENTAL

The investigations involved sampling of municipal domestic and industrial wastewaters that were supplied to public utility Chernihivvodokanal and sampling of biofilms (primary bioaccretions) formed on the surface of water inlet grids. Samples were drawn from July 2016 to August 2017, every three months. This periodicity is determined by the fact that a significant change of the wastewater composition and the temperature regime during the water treatment can occur within the specified time interval.

The analysis of wastewater samples was conducted with due regard for the requirements of existing standards [13]. Indices BOD and COD were determined by the iodometric titration method and dichromate method, respectively. Suspending of the substance and dry residue were determined by the gravimetric method. The pH value was measured potentiometrically using an I-160MI ionometer. Concentrations of Fe^{2+} and Fe^{3+} (joint presence), NH_4^+ and PO_4^{3-} were determined by the photocolorimetry method using a KFK-2 photoelectric colorimeter; and the concentration of chloride ions and sulfate ions were determined by the Mohr argentometric method and complexometric method, respectively, [14].

The number of bacteria (in selected biofilm samples and water samples) were calculated by the method of tenfold limiting dilutions after bacterial inoculation using appropriate liquid selective media: SRB (Postgate's B medium), IRB (Kalinenko's medium), DNB (Giltay's medium), AB (beef-extract broth), NiB of genus *Clostridium* (Winogradsky's medium), and TB (Beyerinck's medium) [15, 16].

SRB enrichment cultures were obtained by inoculation into the Postgate B liquid nutrient medium using samples of biofilms. Bacteria were cultured at temperature 301 ± 1 K in a thermostat during 5–14 days depending on the ecologo-trophic group of bacteria. The bacterial count was calculated by using the Mac-Cready tables [17].

The model corrosion investigations of St-3 grade low-carbon steel under conditions of microbial corrosion were conducted for estimating the SRB corrosion activity. Initially, the ground steel plates having dimensions $4.8 \times 1.5 \times 0.2$ cm were weighed on the analytical balance (VLR-200), disinfected with alcohol, treated with the 3M sulfuric acid solution for removing oxide films and activation of electrochemical processes. These plates were placed into 50 cm^3 flasks filled with corrosive medium (Postgate's B medium) that was inoculated (10% of volume) with an appropriate SRB enrichment culture, the initial titer of which amounted to 10^6 cell/cm^3. The flasks were hermetically sealed by rubber stoppers and placed into a dry-air thermostat (TSO-1/80 SPU), where the temperature was maintained at the level 301 ± 1 K. The duration of exposure was 240 h. For control we used the sterile nutrient medium (Postgate's B) and the same medium but inoculated with the collection bacteria cultures: *Desulfovibrio sp*. 10 (UKM V-11503), *Desulfovibrio vulgaris* DSM644 (UKM V-11501), and *Desulfomicrobium sp*. TC4 (UKM V-11506). The cultures of bacteria were obtained from the Ukrainian collection of microorganisms at the D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine.

After the exposure, steel plates were placed into liquid for 40 min for the removal of corrosion products, then they were washed with distilled water, dried and again weighed on analytical balance. The rate of steel corrosion (g/(cm^2·h)) was calculated on the basis of the plate weight loss. In addition, we analyzed the corrosive medium by determining the accumulation of hydrogen sulfide and protein synthesized by bacteria cells. The hydrogen sulfide concentration in corrosive medium was determined by the iodometric titration method [18]. For determination of protein, the bacteria cells were separated from the culture liquid using the Eppendorf centrifuge with rotor 5415R at 8000 rev/min during 20 min. Next, cells were sonicated on the UZDN-2T ultrasonic disintegrator three times by 2 min with interval 1 min; the residues of cell walls were precipitated by centrifuging, then the protein content was determined in supernatant by the Lowry method [19].

Statistical processing of experimental data and the correlation analysis of corrosive, metabolic, physicochemical, and microbiological indices of wastewater and the count of components of microbial communities were performed using the Excel software package (MS Office 2010). The regression equation was calculated for the relationships with the approximation reliability $R^2 > 0.5$. All tests were repeated three times.

RESULTS AND DISCUSSION

Wastewater treatment plants, in particular the metal surface of equipment at the initial stage of water treatment, are an attractive ecological niche for the development of corrosive microbial community that is stipulated by the concentration of sulfate ions in the range 10–30 mg/dm^3, the values of oxidation–reduction potential in the range 50–(–100) mV, and the values of neutral pH medium 7–7.5 [2].

Our earlier studies of the number of main groups of bacteria developed in biofilms on metal surfaces of wastewater treatment plant showed that the biofilm formed at water inlet grids is characterized by a high SRB count (10^7–10^8 cell/cm^3) [20, 21]. This is the evidence of a formation and development of corrosive microbial community that stipulates the need of subsequent detailed investigation of corrosive biofilms with due regard for seasonal dynamics of physicochemical and microbial indices of wastewater.

In accordance with the performed physicochemical analyses, wastewaters are considerably polluted that is indicated by high indices of BOD and COD (Table 1). This study involved the investigation of values of dry residue characterizing the total content of inorganic and organic substances in water. The dry residue visually was of gray-black color that could indicate the presence of metal sulfides, in particular iron sulfide. According to data from paper [22], the composition of dry residue of wastewater from the municipal sewage treatment plants can include ammonium nitrogen, mineral phosphorous, sulfates, biogenic organic substances, etc. In winter period (February 2017) the value of dry residue was the highest during the entire period of observation and reached the level of 710 mg/dm^3. In summer months (July 2016, August 2017) the amount of dry residue dropped by 40–161 mg/dm^3. Data on the content of Cl^-, NH_4^+, PO_4^{3-}, SO_4^{2-}, Fe^{2+}, and Fe^{3+} in water presented in Fig. 1 indicate the maximum value of dry residue in winter period that is stipulated by an enhanced total concentration of dissolved salts in wastewater. The same behavior depending on the season of sampling was observed for BOD_{tot} index.

Table 1. The indices of wastewater pollution

Date of sampling	Suspended substances	Dry residue	BOD_5	BOD_{tot}	COD	pH
	mg/dm^3					
July 2016	196	670	465.3	586.3	903.3	7.14
February 2017	244	710	490.0	617.4	776.0	7.22
May 2017	224	695	392.7	494.8	816.0	7.16
August 2017	248	549	311.3	392.3	489.3	7.51

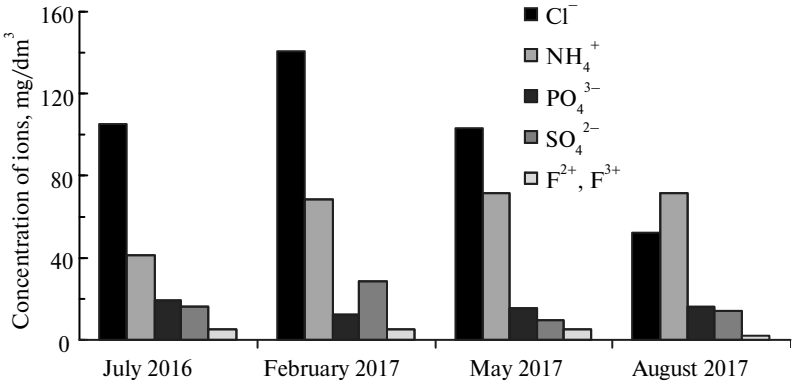

Fig. 1. Dynamics of the ionic composition of wastewater fed to wastewater treatment plants.

The ionic composition of wastewater, in particular concentrations of sulfate and chloride ions and ammonium nitrogen, varied depending on the year season (see Fig. 1). The attention is drawn to the content of Cl^- ions in wastewater and the fact these ions are dominant in wastewater. Their quantity in summer period (July 2016) amounted to 104.9 mg/dm^3, in winter period (February 2017) this quantity increased to 140.4 mg/dm^3, and in spring-summer period (May–August 2017), this figure dropped from 103.3 to 52.5 mg/dm^3. According to data in paper [23], the wastewater may contain bacteria representing facultative anaerobes reducing oxygen-containing chlorine compounds (chlorates and perchlorates) that can be used as electron acceptors. Chloride ions are the final product of this process that along with other factors can contribute to enhancing their concentration in wastewater.

The content of total sulfates in wastewater also varied depending on the season: in summer period (July 2016) it was 16.0 mg/dm^3, in winder period (February 2017) the content increased to 28.3 mg/dm^3, and in spring-summer period (May–August 2017) this content was lower than in winter period and amounted to

10.0–14.0 mg/dm^3. As is known [2], sulfates are the main electron acceptor for SRB, while their concentration is one of the determining factors that limit the development of this group of microorganisms.

The concentration of phosphate ions also fluctuated depending on the year season: in winder period (February 2017) it was low and equal to 12.1 mg/dm^3 and it summer period (July 2016 and August 2017) it increased to the levels of 19.6 and 15.9 mg/dm^3, respectively. It should be noted that SRB in case of the shortage of sulfates can reduce phosphates. In this case, according to data [24], phosphates can be electron acceptors.

Variations of the above water pollution indices can be directly or indirectly related to variations of the microbial composition of wastewater. Therefore we investigated the composition and the count of main ecologo-trophic bacteria groups of microbial community of biofilm formed on the surface of wastewater treatment plants. The dynamics of the bacterial count of bacteria entering belonging to the composition of corrosive microbial community is presented in Fig. 2.

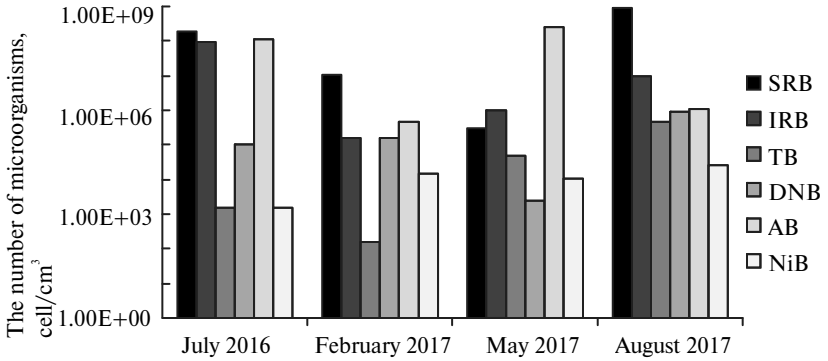

Fig 2. Dynamics of the count of main bacteria groups of sulfidogenic microbial community in biofilm formed on the surface of wastewater inlet grid.

During the entire period of observations (July 2016–August 2017), SRB were dominant in corrosion active microbial community (the bacterial count was up to 10^9 cell/cm^3 of water) that can stipulate in future the manifestation of corrosion activity of biofilms. The bacterial count of the specified group during the period of observations varied from 10^6 to 10^9 cell/cm^3. A considerable amounts of IRB (from 10^5 to 10^7 cell/cm^3) and AB (from 10^5 to 10^8 cell/cm^3) were found in biofilm. DNB were detected in the amount of 10^2–10^6 cell/cm^3. TB and NiB in biofilm were presented in the minimum amounts, and the largest fluctuation of the bacterial count was noted just for TB (10–10^5 cell/cm^3), the NiB count amounted to 10^3–10^4 cell/cm^3.

As regards seasonal fluctuations, it was noted that water samples drawn in summer periods (July 2016, August 2017) contained SBR in the amounts by two–three orders higher (10^8 and 10^9 cell/cm^3, respectively) as compared to the winter-spring period (10^6–10^7 cell/cm^3) in February–May 2017. Similar results were obtained for IRB: in winter period (February 2017) the detected amount was less and amounted to 10^5–10^6 cell/cm^3, while in the spring-summer period their amount increased to 10^6–10^7 cell/cm^3. In February 2017 we observed the smallest titer of tionic bacteria equal to 10^1–10^2 cell/cm^3, while in summer periods (July 2016 and August 2017) the bacterial count of this group of bacteria increased to titers equal to 10^3–10^5 cell/cm^3. Two groups of bacteria showed a higher titer in winter period (February 2017), namely, denitrifying bacteria (10^5–10^6 cell/cm^3) and fermentive type bacteria (10^4–10^5 cell/cm^3), as compared to the spring-summer period. The bacterial count of ammonifying bacteria in summer period (August 2017) and winter period (February 2017) were practically the same and amounted to 10^5–10^6 cell/cm^3, but in July 2016 and May 2017 their numbers significantly increased and reached 10^8 cell/cm^3 that was commensurable with the SRB count. Such spikes of the bacterial count of ammonifying bacteria can be explained by the variation of the organic matter content in wastewaters fed to the wastewater treatment plants, the COD index in July 2016 amounted to 903.3 mg/dm^3, while in May it was 816 mg/dm^3.

The composition and bacterial count of bacterial groups of corrosive microbial community agree with data from papers [2, 6], while their seasonal dynamics and the ratio in corrosive microbial community in tested biofilm samples can indicate the stable system supported by trophic ties between the detected groups of microorganisms [25, 26].

The investigation of metabolic activity of SRB enrichment cultures made it possible to establish that their largest amount was registered in summer months (Fig. 3). A distinct relationship between the hydrogen sulfide production and SRB count was not detected. However, an inverse relationship between the amount of SRB and the concentration of total sulfates in wastewater was revealed, because sulfates are the main acceptor of electrons for the specified group of bacteria (see Fig. 3a). We also discovered the relationship between the bacteria produced hydrogen sulfide and the measure of wastewater acidity. So, in July 2016 the production of hydrogen sulfide amounted to 513.4 μg H_2S/cm^3, while in winter months the metabolic activity of bacteria was less (in February 2017 it amounted to only 88.0 μg H_2S/cm^3). It should be noted that the largest pH value (7.51) was observed in the wastewater sample drawn in August 2017. This value increased by 0.29 as compared to February 2017. A high production of hydrogen sulfide by bacteria of microbial community resulted in the variation of wastewater pH value in the direction of acidulation that could negatively affect the metal structures of wastewater treatment plants.

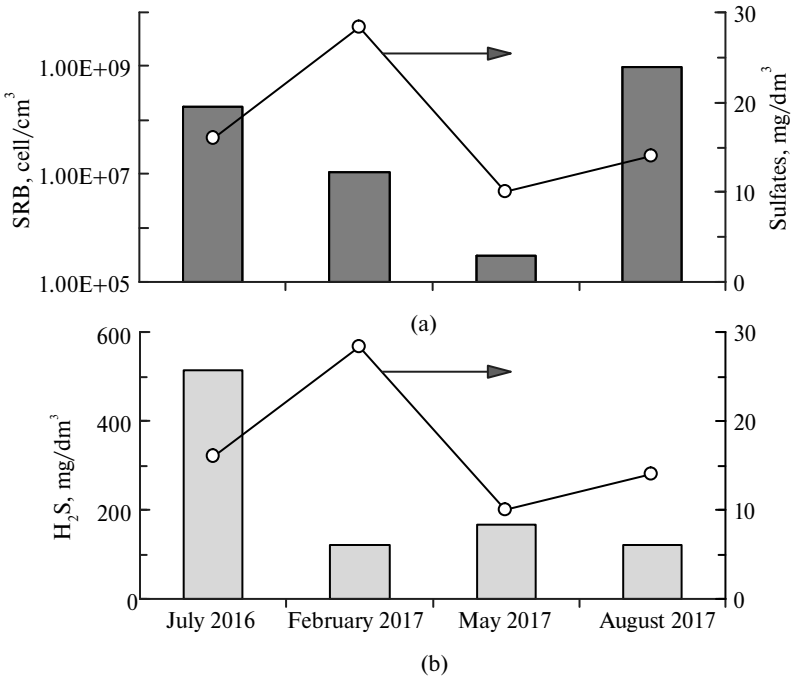

Fig 3. Metabolic activity of sulfate-reducing bacteria: bacterial count (a); hydrogen sulfide production (b).

Next, the determination of the seasonal effect on the corrosion activity of SRB involved the need to separate the following enrichment cultures of bacteria from the samples of biofilm accretions: Ch1 (July 2016), Ch3 (February 2017), Ch5 (May 2017) and Ch7 (August 2017). In addition, model tests for determination of the metabolic and corrosion activity of SRB in forming and functioning of biofilm on the surface of steel plates (Table 2) were performed.

Table 2. Metabolic activity of enrichment cultures of sulfate-reducing bacteria separated from the wastewater biofilms

SRB (date of biofilm sampling)	SRB titer, $cell/cm^3$		Synthesis of protein, $\mu g/cm^3$	Production of H_2S, mg/dm^3
	initial	final		
Ch1 (July 2016)	7.5×10^7	9.5×10^9	201.66	302.6
Ch3 (February 2017)	2.5×10^6	1.1×10^9	1416.67	482.0
Ch5 (May 2017)	4.5×10^6	1.5×10^8	586.67	448.4
Ch7 (August 2017)	1.5×10^6	9.5×10^9	506.65	519.7

The titer of bacteria separated in different year seasons during the model test for determining the corrosion rate was not identical: the initial titer of bacteria amounted to 1.5×10^6–7.5×10^7 $cell/cm^3$, after culturing with steel samples the final titer increased by two-three orders of magnitude (1.5×10^8–9.5×10^9 $cell/cm^3$).

The protein synthesis data indicated that the largest value (1416.67 μg/cm^3) was observed for culture Ch3 in February 2017, while the smallest value (201.66 μg/cm^3) was observed for culture Ch1 in July 2016. For cultures Ch5 and Ch7 the indices of protein synthesis amounted to 586.6 and 506.6 μg/cm^3. The comparison of values of hydrogen sulfide production by enrichment cultures of bacteria showed that the high index (519.7 mg/dm^3) was detected for Ch7 in August 2017. The activity of SRB separated from the biofilm samples drawn in February 2017 was below by 7.2, while in May 2017 it was below by 13.7%. The lowest index of hydrogen sulfide production (302.6 mg/dm^3) was obtained for sample Ch1 in July 2016.

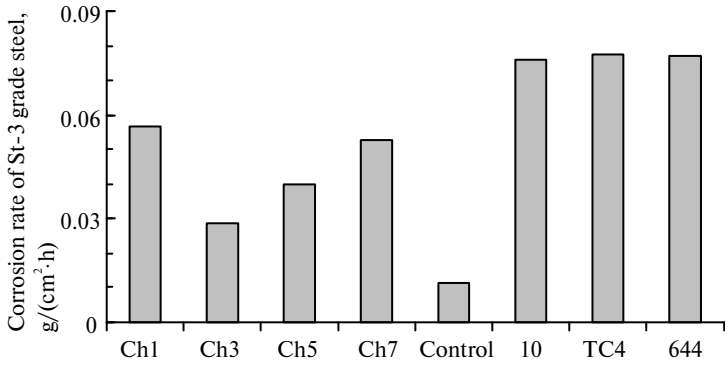

Fig. 4. The corrosion rate of St-3 grade low-carbon steel under the exposure to the enrichment and collection cultures of sulfate-reducing bacteria: Ch1 is the enrichment culture of SRB separated from the biofilm sample in July 2016; Ch3, Ch5, Ch7 are the same culture but separated in February 2017, May 2017, and August 2017, respectively; control is the sterile nutrient medium (Postgate's B); 10 is the collection culture *Desulfovibrio sp. 10*; TC 4 is the collection culture *Desulfomicrobium sp.*; 644 is the collection culture *Desulfovibrio vulgaris DSM644*.

Model tests revealed that the corrosion rate of St-3 grade steel significantly fluctuated in the Postgate B medium inoculated with SRB separated from microbial communities and biofilms formed in different year seasons (Fig. 4). Differences in corrosion rate of steel plates in the presence of microbial communities, probably, are related to fluctuations of the water temperature and metabolic activity of SRB. Bacterial cultures sampled in summer periods (July 2016 (Ch1) and August 2017 (Ch7)) ensured a high rate of steel corrosion, namely, 0.0568 and 0.0528 g/(cm^2·h). The steel corrosion rate under exposure to culture Ch3 (February 2017) was by 58–62% lower (0.0286 g/(cm^2·h)) than for the cultures separated in summer periods. In sterile Postgate's B medium (control) the corrosion rate of St-3 grade steel plates amounted to 0.0112 g/(cm^2·h).

The comparison of corrosion activity of enrichment SRB cultures with corrosion indices of collection SRB cultures *Desulfovibrio* sp. 10, *Desulfomicrobium* sp. TC4 and *D. vulgaris* DSM644 makes it possible to see that the activity of the latter is practically twofold higher (see Fig. 4). The tested SRB cultures were less corrosion active than the collection cultures. However, the rate of steel corrosion under exposure to microbial community of wastewater may be affected by numerous environmental factors that can lead to the change of potential corrosive microbial community into a corrosion aggressive one. Therefore the dominance of corrosive (corrosion active) microorganisms in the composition of wastewater microbiota at wastewater treatment station is undesirable.

For the purpose of revealing factors that can materially affect the microbial community and, consequently, lead to changes of its metabolic and corrosion activity, the correlation analysis of relationships of the corrosion activity index of SRB enrichment cultures depending on the data of biofilm microbial analysis (the bacteria group count) and the results of physicochemical analysis of water, in particular, the concentration of ions in wastewater (Table 3).

The obtained data made it possible to establish a direct correlation between the rate of steel microbial corrosion and the IRB count in microbial community of biofilm and also the concentration of phosphate ions in wastewater. In this case, the probability of the influence of phosphate ions and IRB count on corrosion processes amounted to 0.99 and 0.796, respectively. In accordance with paper [27], phosphate ions are reduced by microorganisms to phosphide ions, the interaction products of which with metal iron do not possess protective properties, i.e., they do not form protective films on the surface of metals that results in cathode depolarization, unlike the interaction products of the main SRB metabolite (hydrogen sulfide).

The increased production of hydrogen sulfide by microbial community and increased concentration of ammonium ions in wastewater can lead to an enhanced rate of steel corrosion with correlation indices 0.694 and 0.64, respectively. At the same time, correlation indices of the corrosion rate versus SRB count relation-

ship were insignificant and equal to only 0.026 that could indicate the corrosion activity of SRB in wastewater related to indirect impact of bacteria, namely, the production of corrosion active metabolite (hydrogen sulfide).

Table 3. Correlation indices for the relationships of the corrosion rate of St-3 grade steel depending on the microbiological and chemical factors

Factors	R^2	Correlation equation of the form $y = bx + a$ for $R^2 > 0.5$
PO_4^{3-}	0.9917	$y = 3.8E - 3x - 0.0177$
IRB count	0.7965	$y = 2E - 10x + 0.0358$
H_2S production	0.6941	$y = -6E - 05x + 0.0673$
NH_4^+	0.6418	$y = -6E - 4x + 0.082$
NiB count	0.3336	–
SO_4^{2-}	0.3044	–
Zn^{2+}	0.1475	–
Cl^-	0.1366	–
Cr^{3+}	0.1245	–
AB count	0.0838	–
SRB count	0.0266	–
Ni^{2+}	0.0053	–
Fe^{2+}, Fe^{3+}	0.0037	–

Note. Values of R^2 for the bacterial count of tionic, denitrifying bacteria and Cu^{2+} concentrations were insignificant and amounted to < 0.001.

CONCLUSIONS

It has been shown that the corrosion active biofilm is formed on metal surfaces of wastewater treatment plants irrespective of the year season.

The sulfate-reducing and ammonifying bacteria (10^9 cell/cm^3 and 10^7–10^8 cell/cm^3, respectively) are dominant in the film, while acidophobic tionic bacteria belong to the group with the smallest bacterial counts (up to 10^2 cell/cm^3). The relationship between the hydrogen sulfide produced by sulfidogenic community and the variation of wastewater pH value was established. So, the rise of the hydrogen sulfide amount produced by SRB resulted in the reduction of wastewater pH from 7.51 to 7.22. According to data of correlation analysis, the corrosion activity of sulfidogenic microbial community of wastewater biofilm can be influenced by chemical factors such as the concentration of phosphate ions and ammonium ions, and also by microbiological factors such as the bacterial count of iron-reducing bacteria and production of hydrogen sulfide by sulfate-reducing bacteria.

Thus, it is important to provide methods and techniques for anti-corrosion protection of metal constructions susceptible to the negative effect of corrosive microbial community for the purpose of ensuring the stable operation of wastewater treatment facilities. The obtained results can be used for the development of recommendations aimed at the creation of measures for preventing the break-up of wastewater treatment facilities and provision of anti-corrosive protection of constructions with due regard for the effect of microbiological factor.

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