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Role of Polymer Complexes in the Formation of Biofilms by Corrosive Bacteria on Steel Surfaces

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Abstract—The composition of exopolymer complexes (EPCs), synthesized by the monocultures *Desulfovibrio* sp. 10, *Bacillus subtilis* 36, and *Pseudomonas aeruginosa* 27 and by microbial associations involved in the corrosion of metal surfaces has been studied. An analysis of the monosaccharide composition of carbohydrate components, as well as the fatty acid composition of the lipid part of EPCs, was carried out by gas–liquid chromatography (GLC). It was found that bacteria in biofilms synthesized polymers; this process was dominated by glucose, while the growth of bacteria in a suspension was marked by a high rhamnose content. Hexouronic acids and hexosamine have been revealed as a part of *B. subtilis* 36 and *P. aeruginosa* 27 EPCs. Qualitative differences were revealed in the fatty acid composition of exopolymers in biofilms and in a bacterial suspension. It was shown that the transition to a biofilm form of growth led to an increase in the unsaturation degree of fatty acids in the exopolymers of associative cultures. The results can be used to develop methods to control microbial corrosion of metal surfaces.

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INTRODUCTION

In recent decades, studies have shown that in natural ecosystems the majority of bacteria exist in the form of specifically organized biofilms attached to substrates [1–3]. Biofilms are a highly organized structured community produced by bacteria of one or more species [3, 4]. A microbial community functions in a biofilm as a coordinated consortium, immersed in the exopolymer matrix of synthesized (by itself) extracellular components: polysaccharides, lipids, proteins, nucleic acids, and other substances [4–6]. Bacterial exopolymers play an important role not only in the formation of a biofilm structure, but also in its interaction with the surface, at which it develops. Biofilm formation is always accompanied by a modification of the surface and changes in the properties of bacteria in the community [2, 6].

The development and functioning of microbial communities in biofilms are not always taken into account properly when assessing their role in the environment, particularly, in areas subjected to an anthropogenic impact, such as areas of underground communication installations (heating mains and oil and gas pipelines).

We have previously shown that in the ground, which is in direct contact with the surface of a pipe, a sulfidogenic microbial community, contributing to the emergence of biocorrosion foci, is formed [7]. Sulfate-reducing bacteria, which participate directly in the bioelectrochemical processes occurring at the surface of the metal in a biofilm, play the main role in the microbial corrosion of steel [7–9]. We found that, in addition to sulfatreducers, bacteria of the genera *Bacillus* and *Pseudomonas* are dominant members of such a sulfidogenic community [10].

However, despite the numerous works devoted to biofilm research, there is little information in today's literature on the formation of biofilms on steel by corrosive microbial communities and on the role of exopolymer complexes (EPCs) in the corrosion process.

The purpose of this work is to study the composition of EPCs, synthesized by mono- and associative cultures isolated from a natural sulfidogenic community, and to define the role of its dominant members in the formation of biofilms at steel surfaces.

MATHERIALS AND METHODS

Objects of study. We used *Desulfovibrio* sp. 10, *Bacillus subtilis* 36, and *Pseudomonas aeruginosa* 27 bacteria isolated from a biofilm formed on steel-3 [10], as well as an association artificially created by the above-mentioned monocultures. To create the association, cultures of the bacteria *Desulfovibrio* sp. 10, *B. subtilis* 36, and *P. aeruginosa* 27, grown in Postgate's B liquid medium [11], were used in the exponential growth phase. The suspensions of bacteria were mixed

at a ratio of 1 : 1 : 1. The natural sulfidogenic bacterial association, which we had earlier selected from the soil at the surface of the tube of the existing pipeline in the Carpathians, was grown in Postgate's B medium.

A study of the bacteria in a biofilm and in a bacterial suspension,

formed on steel plates, was carried out in a laboratory experiment. The study was performed in 500-ml bottles filled with Postgate's B nutrient medium and inoculated, depending on the variant of the experiment, with one or more cultures of bacteria in the exponential growth phase. The amount of the inoculum was 10% of the volume of the medium; the initial titer of monocultures was 10^7 cells/ml. Hanging on a fishing line, steel-3 samples (4.8 × 1.5 × 0.5 cm in size), which had been preweighed, sterilized, and treated with 6N H_2SO_4 to remove oxide films and intensifying electrochemical processes, were immersed in the bottles [12]. Then the bottles were closed tightly with rubber stoppers and incubated at 28°C for 90 days. Each experiment was conducted three times. The accumulation of biomass (as protein) and hydrogen sulfide was an indicator of bacterial growth. The content of the cell protein was determined by the Lowry method. For this purpose, the biofilm and plankton cells were sonicated three times for 2 min at intervals of 1 min on a UZDN–2T device (Soviet Union) and centrifuged to precipitate cellular walls at 7000 g for 30 min; the supernatant was decanted, and its protein content was measured. The accumulation of hydrogen sulfide in the medium was determined by iodometric titration [13].

Isolation of the EPC. To isolate the exopolymers produced by the biofilm cells, steel-3 samples were removed from the culture medium and immersed in 100 ml of 0.1 M phosphate buffer (pH 7.0). The biofilm was removed from the samples at 22 kHz ultrasound (30 s); the treatment was performed twice at an interval of 30 s on a UZDN-2T device (Soviet Union). To separate the cells, the resulting suspension was centrifuged at 15000 g and 20°C for 40 min. The supernatant was dialyzed in distilled water for 3 days [14]. The resulting dialysates were evaporated on a rotary evaporator (Heidolph, Germany) of up to 50 ml to concentrate the exopolymer solution and then lyophilized. The amount of protein in the cells, precipitated after centrifugation, was determined as described above. After the removal of the biofilm, the steel samples were treated with the following solution to remove the corrosion products: 84 g of sulfuric acid, 100 g of ammonium citrate dibasic, 10 g of thiourea, and 880 ml of distilled water. The exposure period was from 10 to 40 min, and then the samples were washed with distilled water, dried with filter paper, and weighed. The bacterial corrosion activity was determined based on the reduction in the steel mass compared to the original surface area (per unit) of the plate per unit time [12].

The EPC produced by the cells in the suspension was isolated from the supernatant after centrifugation at 15000 g and 20°C for 40 min as described above.

Monosaccharide composition of the EPC. The monosaccharide composition was determined by gas–liquid chromatography. For the analysis, the samples were pretreated, according to the procedure in [15]; 2N HCl (0.4 ml) was added to the lyophilized preparation (2 mg). The EPC mixture was sealed in a vial, and then hydrolysis was performed for 4–5 h at 100°C. The hydrolyzate was evaporated to dryness and washed three times with distilled water on a rotary evaporator at 45–50°C. The sample was resuspended in 1 ml of distilled water, and sodium borohydride was added. The samples were left in darkness for 12 h; then they were neutralized in the H^+ form with a KU-2 cation exchanger. The cationite was removed by filtration on a glass filter no. 4 with a pore size of 5–15 μm, and the filtrate was evaporated to dryness; washed three times with methanol; evaporated on a rotary evaporator at room temperature; and, after adding pyridine (0.5 ml) and acetic anhydride (0.5 ml), was kept at 100°C for 15 min. The resulting mixture was evaporated to dryness in several stages by adding methanol and distilled water. The samples were resuspended in chloroform (2–3 ml) and centrifuged at 5000 g for 20 min. The supernatant was evaporated to dryness.

An analysis of neutral sugar derivatives in the form of their full acetates of polyols was carried out on a 6890N gas chromatograph with a 5973 inert mass spectrometry detector (Agilent Technologies, United States). A DB–225ms capillary column (J&W Scientific) 30 m × 0.25 mm × 0.25 μm in size was used. Separation by chromatography was carried out in isothermal mode at 220°C: the carrier gas was helium, and the flow rate through the column was 1.0 ml/min. The evaporator temperature was 250°C, sample injection was carried out with a division of the flow (1 : 100), and the temperature of the GC/MC interface was 280°C. Detection was carried out in scanning mode within the range from 100 to 600 m/z.

The identification of acidic and neutral sugars was carried out in the form of their acetylated methylglycosides, which were analyzed on an HP-5MS column with the following temperature program: 150°C, 5 min; from 150 to 250°C, by 3°C for 1 min; and 250°C, 10 min [16].

Determination of the fatty acid composition of the lipid part of the EPC. A mixture of methylene chloride and methanol (2 : 1) in the volume of 1.5 ml was added to a freeze-dried EPC (5–10 mg); following vigorous shaking, it was left for 12 h. Next, after adding 2 ml of water, it was shaken for 10 min, allowed to phase separate, and the lower phase (0.5 ml) was collected. It

Table 1. Metabolic activity and corrosiveness of bacterial monocultures and their communities

Bacterial culture	Protein in the cells, μg/ml		Accumulation of hydrogen sulfide, mg/ml	Corrosion rate, g m^{-2} h^{-1}
	biofilm	suspension		
Natural association	1720	159	332 ± 5.1	0.239 ± 0.01
Artificial association	1800	100	286 ± 4.8	0.178 ± 0.003
Desulfovibrio sp. 10	1880	200	291 ± 4.4	0.307 ± 0.01
P. aeruginosa 27	75	690	48 ± 2.3	0.086 ± 0.0035
B. subtilis 36	48	485	23 ± 0.6	0.072 ± 0.0022

was transferred to a 5-ml vial, evaporated to dryness on a rotary evaporator at 40°C, dried for 2 h in a vacuum desiccator over KOH, and then 1 ml of 4 M HCl in methanol was added to it. The vial was sealed, left for 4 h at 80°C, transferred to a flask, and evaporated to dryness; the second time, with 2 ml of methanol. A saturated solution of sodium chloride in water (1 ml) was added to the dry residue. Then the extraction, being stirred well, was twice extracted in 2 ml of chloroform. The lower phase was removed and evaporated to dryness. Hexane in the amount of 1.5 ml was added, and the sample was transferred to a container for analysis. A GLC–MS analysis was performed on a HP-5 MS capillary column 30 m × 0.25 mm × 0.25 mm in size: 5% phenyl methyl siloxane was used, the temperature was from 150 to 210°C (4°C/min), the carrier gas was helium, the flow rate was 1 ml/min, and a 6890N gas chromatograph with a 5973 inert mass spectrometric detector (Agilent Technologies, United States) was used [17].

RESULTS AND DISCUSSION

Under natural conditions, the most corrosive sulfate-reducing bacteria usually develop in a microbial community, each member of which may play a role in biofilm formation [7–9]. Proceeding from this fact, our researches have been focused on the study of biofilm formation by both a community and the *Desulfovibrio* sp. 10, *B. subtilis* 36, and *P. aeruginosa* 27 monocultures, which are permanent components of the community.

Observations showed that after exposure of steel specimens to a medium with bacteria a biofilm was formed on its surface. The development of bacteria was evaluated by the accumulation of biomass both in the environment and in the biofilm (Table 1). It was found that sulfidogenic associations and the *Desulfovibrio* sp. 10 monoculture developed much better in the biofilm. The amount of cellular protein in the biofilm after exposure was by an order of magnitude higher compared to the suspension. The large accumulation volume of biomass in the biofilm may be related to the ability of sulfate-reducing bacteria to positive chemotaxis to iron [7]. In addition, the presence of Fe^{3+} cations on the surface of a substrate significantly increases the number of adhered *Desulfovibrio* sp. anaerobic bacteria [3]. It is the chemotactic for Fe^{3+} and adhesion on metal surfaces that promote the concentration of these bacteria in a specific econiche, where, due to electrochemical processes, accumulation of metal ions occurs and a corrosion-aggressive microbial community forms. Heterotrophic satellites of sulfate-reducing *B. subtilis* 36 and *P. aeruginosa* 27 synthesized significantly less protein in the biofilm, but the accumulation of biomass in the suspension significantly increased.

The production of hydrogen sulfide indicated the growth of bacteria, as well as their corrosion aggressiveness. During cultivation of sulfidogenic associations and *Desulfovibrio* sp. 10, the amount of hydrogen sulfide was 286–332 mg/l. The accumulation of hydrogen sulfide was correlated with the corrosion damage of steel specimens, which amounted to 0.307 g $m^2 \cdot h^{-1}$ for the *Desulfovibrio* sp. 10 monoculture. This proves that hydrogen sulfide formed during the recovery of sulfur from the sulfate medium ($S^{+6} \rightarrow S^{-2}$) may be directly involved in the corrosion process. The interaction between hydrogen sulfide and iron ions leads to the formation of iron sulfide, which serves as an additional cathode amplifying electrochemical processes [9, 18]. In the culture medium with *P. aeruginosa* 27 and *B. subtilis* 36, a small amount of hydrogen sulfide and a low corrosiveness level were found. The corrosion rate in the presence of these bacteria was 2–4.3 times lower than with the participation of sulfatreducers.

The corrosion aggressiveness of a microbial community is determined by the ability of the biofilm they

form to catalyze electrochemical processes on the metal surface, causing its destruction [19, 20]. An important corrosion factor in the area of biofilm interaction with metal is the exopolymer matrix, in which the bacteria and extracellular polymers synthesized by them are immersed, i.e., the so-called EPC [8, 20]. However, the role of EPC components in the overall picture of biocorrosion is currently not well determined. It is known that exopolysaccharides, playing a key role in the adhesion and formation of the biofilm structure, are the dominant component of the EPC [2, 6]. During the interaction of bacteria with a corrode metal, the produced exopolysaccharides, due to their polyanionic properties, contribute to the consolidation of the corrosion products in the biofilm [20]. On the other hand, there are data that exopolysaccharides of some bacteria can form dense films on metal surfaces, significantly inhibiting the corrosion process [21]. It is advisable to carry out a comparative study of the synthesis of the EPC carbohydrate components by mono- and associative cultures of bacteria for different growth models: in a biofilm and in a culture medium.

We found that the composition of the EPC carbohydrate components varied, depending on both the type of the bacteria producing it and the growth model.

A characteristic feature of the EPCs of both the biofilm and the cell suspension was the presence of neutral carbohydrates—glucose, galactose, mannose, rhamnose, fucose, and xylose—in the monosaccharide composition. In the biofilm formed by the natural sulfidogenic association and the monocultures of *P. aeruginosa* 27*, B. subtilis* 36, except for the above carbohydrates, ribose and arabinose were found. The latter was also synthesized by *Desulfovibrio* sp. 10 suspension cells (Table 2).

In the monosaccharide composition of the biofilm, exopolymer glucose was dominated, the amount of which reached 25.2–36.4%. The structure of the biofilm matrix is mainly stabilized by polysaccharides; their monomers are more likely to be hexoses, particularly, glucose [6, 21].

Glucose was also dominated in the EPC in the medium; the content ranged from 17.5 to 31.4%. A higher content of rhamnose, which may be located in an EPC in the form of rhamnose residues in polysaccharide, glycoproteins, and glycolipids, was noted in the medium in comparison with the biofilm. The largest amount of rhamnose (24%), which is a typical component of glycolipids, was produced by cultures of the heterotrophs *P. aeruginosa* 27, *B. subtilis* 36 during steady growth. These bacteria are producers of rhamnolipids, which in the structure of a biofilm may play a significant role in its formation [22]. This assumption is supported by studies of the synthesis of rhamnolipids in *P. aeruginosa* biofilms. The authors of [23] showed that the production of *P. aeruginosa* rhamnolipids is necessary to prevent the tight attachment and adhesion of bacteria within a biofilm, which promotes the formation of channels through which oxygen and products of metabolism circulate in the biofilm.

According to studies [6, 20], the structure of a biofilm largely depends on the content of monosaccharide units with charge in polysaccharides, namely, uronic acids (galacturonic and glucuronic) and amino sugars (galactosamine and glucosamine). Therefore, we focus on the content these particular monomers in EPCs. The greatest variety of uronic acids and amino sugars has been found among exopolymers of sulfate-reducing heterotrophic satellites: *P. aeruginosa* 27*, B. subtilis* 36. In the *P. aeruginosa* biofilm, the volume of uronic acids and amino sugars was 4.6 and 1.6 times more (respectively) than in the suspension of bacteria. Galactosamine and glucosamine were also found in the exopolymers synthesized by the associative cultures. Only galactosamine was found among the *Desulfovibrio* sp. 10 exopolymers. A comparison of the monosaccharide composition of the EPCs of the *Desulfovibrio* sp. 10, *P. aeruginosa* 27, and *B. subtilis* 36 monocultures clearly shows that it is heterotrophic assiociants of sulfate-reducing bacteria that produce polysaccharides, which are found to contain the greatest variety of neutral carbohydrates, uronic acids, and glucosamine. They are known to interact with other exopolymer components through carboxyl and amino groups, contributing to the formation of biofilms and to the strengthening of their structures [2, 14]. In addition, polysaccharides synthesized by bacteria are able to bind metal ions and sulfides in a matrix, enhancing the corrosion process [20].

In recent years, data have been obtained on the significant role of minor components of the EPC, namely, lipids and fatty acids, in the formation and functioning of biofilms [24–27]. A study of the *Acidithiobacillus ferrooxidans* EPC showed that a significant part of it is formed by fatty acids, which, according to the authors of [24], are involved in cell adhesion to sulfide minerals. In the biosynthesis of EPCs, fatty acids are able to covalently bind to hydrophilic molecules, forming surface-active compounds: glycolipids (ramnolipids) and lipopeptides. On the one hand, this synthesis allows bacteria to adhere to hydrophobic substrates [25], and, on the other hand, these compounds are responsible for important stages in the formation of specific biofilm structures, namely, biofilm channels in the matrix, through which the active transport of oxygen, water, dissolved nutrients agents, inhibitors, and other compounds occurs [22, 26].

Given the above-mentioned, we isolated the lipid component of the EPC of mono- and associative cultures and determined its fatty acid composition.

Table 2. Monosaccharide composition of the EPC of monocultures and their communities for different growth models

Bacterial culture	Growth model*	Monosaccharides, %**											
		hexoses			deoxyhexoses		pentoses			hexuronic acids		hexosamines	
		glucose	galactose	mannose	rhamnose	fucose	xylose	ribose	arabinose	galacturonic	glucuronic	galactosamine	glucosamine
Natural association	BF	33.41	8.79	9.51	8.20	14.56	2.62	2.20	1.67	–	–	10.40	4.79
	BS	31.36	9.28	15.75	13.42	17.29	3.89	3.74	–	–	–	11.32	–
Artificial association	BF	36.40	3.47	5.89	3.21	20.39	2.15	–	–	–	7.42	3.27	–
	BS	19.63	17.99	16.81	8.77	14.46	3.32	4.22	–	–	–	14.73	1.32
Desulfovibrio sp. 10	BF	34.17	4.76	4.95	3.83	19.16	2.46	–	–	–	–	2.66	–
	BS	25.19	17.41	26.99	9.76	5.92	2.31	2.90	1.24	–	–	8.40	–
Pseudomonas aeruginosa 27	BF	33.03	9.33	11.49	7.50	17.02	2.63	0.57	0.23	3.03	4.17	9.28	10.07
	BS	25.21	8.59	20.79	23.72	7.54	2.39	1.17	1.68	1.56	–	10.60	1.25
Bacillus subtilis 36	BF	25.19	6.50	6.79	14.76	18.21	2.92	2.33	1.39	8.42	1.44	5.66	–
	BS	17.47	17.89	30.91	24.12	8.02	2.11	1.20	–	–	–	9.59	0.09

Notes: * BF – biofilm, BS– bacterial suspension, " –" carbohydrates were not found.
** % of the total area of the peaks in the chromatogramå.

Table 3. Fatty acid composition of the lipid part of the biofilm EPC

Fatty acid*	Number of carbon atoms	Natural association	Artificial association	*Desulfovibrio* sp. 10	*P. aeruginosa* 27	*B. subtilis* 36
Dodecanoic	$C_{12:0}$	1.76	1.55	1.35	1.77	1.99
β-hydroxydodecanoic	$C_{12:0\text{-}3\text{-}OH}$	1.75	1.33	1.64	3.98	0
Tetradecanoic	$C_{14:0}$	7.09	6.93	7.14	6.37	7.83
Pentadecanoic	$C_{15:0}$	4.02	4.28	4.37	3.83	4.47
Antiisopentadecanoic	Antiiso $C_{15:0}$	1.74	1.81	2.41	1.81	0
Hexadecanoic	$C_{16:0}$	37.48	35.65	39.06	36.76	47.34
Hexadecenoic	$C_{16:1}$	10.17	12.35	12.19	11.71	7.44
Heptadecanoic	$C_{17:0}$	1.29	1.45	1.18	1.48	0
Octadecanoic	$C_{18:0}$	14.11	13.51	12.69	13.88	19.3
Octadecenoic	$C_{18:1}$	15.44	16.8	15.53	15.53	11.63
Octadienoic	$C_{18:2}$	3.09	3.35	2.45	2.88	0
Docosanoic	$C_{22:0}$	2.06	0.99	0	0	0
Degree of saturation		0.32	0.36	0.33	0.33	0.19

* % of total fatty acids.

As part of the lipid components extracted from exopolymers of biofilms and suspensions of bacteria, saturated and unsaturated fatty acids were found. In the biofilms, saturated hexadecanoic and oktadecanoic acids were predominant (Table 3). The amount of hexadecanoic acid in the EPCs of sulfidogenic associations, *Desulfovibrio* sp. 10, and *P. aeruginosa* 27 was within the range from 35.65 to 39.06%; that of oktadecanoic acids, from 12.69 to 14.11%. In the biofilm formed by *B. subtilis* 36, the amount of the mentioned acids was higher and was equal to 47.34 and 19.3%, respectively. It should be noted that β-hydroxydodecanoic, antiizopentadecanoic, and heptadecanoic acids, which were found in the biofilms of the sulfidogenic *Desulfovibrio* sp. 10 and *P. aeruginosa* 27 associations, were absent in the fatty acid composition of lipid *B. subtilis* 36 biofilm polymers. Unsaturated hexadecenoic (from 10.17 to 12.35%), octadecenoic (from 11.63 to 16.8%), and oktadiene acids (from 2.45 to 3.35%) were also found in the biofilm.

Qualitative differences were detected in the fatty acid composition of the lipid part of the biofilm EPC and the bacterial suspension. For example, in the EPC synthesized by bacteria in a suspension, dodecanoic, hydroxydodecanoic, heptadecanoic, and docosanoic acids, which were present in the biofilm, were not identified. At the same time, the EPC, synthesized by suspension cells, contained cis- and trans-octadecenoic acids not present in the EPC of the biofilm (Table 4).

In the lipid component of the EPCs of both the biofilm and suspension, other fatty acids were identified, but their number was insignificant. In the lipid part of the biofilm EPC, 12 fatty acids were identified; in the suspension, 9 fatty acids. It should be emphasized that the content of unsaturated fatty acids was higher in the biofilm. The degree of unsaturation dur-

Table 4. Fatty acid composition of the lipid part of the EPC of a bacterial suspension

Fatty acid*	Number of carbon atoms	Natural association	Artificial association	*Desulfovibrio* sp. 10	*P. aeruginosa* 27	*B. subtilis* 36
Tetradecanoic	$C_{14:0}$	20.8	0	2.39	1.37	5.27
Hydroxytetradecanoic	$C_{14:3OH}$	2.88	3.32	1.53	1.77	0
Pentadecanoic	$C_{15:0}$	0.5	0	0	0	1.59
Antiisopentadecanoic	Antiiso $C_{15:0}$	1.03	0	0.9	1.45	4.67
Hexadecanoic	$C_{16:0}$	27.25	33.82	27.25	31.49	40.93
Hexadecenoic	$C_{16:1}$	0.67	0	2.84	1.62	0
Octadecanoic	$C_{18:0}$	42.06	34.7	30.03	30.45	27.7
Octadecenoi	$C_{18:1cis}$	15.9	20.7	22.67	19.03	17.55
Octadecenoic	$C_{18:1trans}$	7.6	7.46	12.38	12.81	2.29
Degree of saturation		0.24	0.28	0.38	0.33	0.20

* % of total fatty acids.

ing the transition from the suspension form of growth to growth in a biofilm differed. In the microbial associations, the degree of unsaturation of fatty acids of the biofilm EPC was significantly higher than in the suspension. It is known that unsaturated fatty acids exhibit a greater degree of reactivity and can interact with proteins, polysaccharides, and other polymers. The binding of lipids, particularly, fatty acids, may contribute to the formation of channels in a biofilm and enhance its stability [22]. In addition, the role of certain fatty acids in the "quorum sensing" regulation of biofilms is discussed. In particular, cis-2-decenoic monounsaturated fatty acid was found to be a signal molecule, which is capable of inducing the collapse of biofilm microcolonies. Among the extracellular fatty acids of bacteria, the existence of other signaling molecules is possible [27].

Thus, exopolymers produced by corrosion-aggressive bacteria may contribute to the adhesive, formative, and communicative processes, which contribute to the functioning and formation of the biofilm structure.

Our research on the role of EPCs in the formation of biofilms on steel is the initial stage in modeling microbial corrosion in vivo. An understanding regarding the fundamental and environmental mechanisms of biofilms will help develop a new management strategy to control them, which will facilitate the creation of new methods for protecting metals against biocorrosion damage.

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