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OF THE REPUBLIC OF KAZAKHSTAN
of the Institute of Plant Biology and Biotechnology

**БИОЛОГИЯ ЖӘНЕ МЕДИЦИНА
СЕРИЯСЫ**



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БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ



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INFLUENCE OF BIOCIDES ON GROWTH AND DEVELOPMENT OF CORROSIVE-DANGEROUS MICROFLORA

Abstract. The problem of protection of buildings and constructions from aggressive chemical and biological impacts of the environment becomes very urgent now. Microbiological corrosion is the important factor influencing reliability and durability of steel concrete designs. In this connection, the problem of protecting building constructions and structures from aggressive biological influences is of great urgency. The aim of the research was to study the effect of biocides based on copolymers and copper sulfate on the development of corrosive microorganisms. It has been established that copper sulfate possessed the least effective inhibitory effect on thione and sulfate-reducing bacteria (SRB). When it was added, the growth of the investigated bacteria was suppressed at a concentration of 1%. Copolymers with different mole composition were more active. Their effect on the inhibition of thiobacteria and SRB occurred at concentrations of 0.1-0.5%. The most sensitive to their effects were *T. ferrooxidans* and SRB. On heterotrophic bacteria and fungi, the compounds tested had a bactericidal effect only at a concentration of 1%. Copper sulfate was more effective with respect to micromycetes and yeast, while inhibition of their growth occurred at concentrations of 1% and 0.5%, respectively.

Keywords: biocorrosion, corrosive-dangerous microorganisms, thione and sulfate-reducing bacteria, heterotrophic microorganisms, biocides.

Introduction. Biological corrosion are the processes of damage to metals, metal structures and other building materials caused by the products of vital activity of living organisms that settle on the surface of building structures. Significant role in biocorrosion is played by such microscopic organisms as bacteria and microscopic fungi, for the development and reproduction of which under certain conditions of operation of buildings and structures a favorable environment is created [1].

Information on the role of the microbiological factor in the corrosion of metals and other materials is accumulated every year, generalized, and losses are calculated for the economy. Numerous species of microbial corrosion testifies to the unusually wide spread of this phenomenon in various spheres of human activity [2-5]. The activity of microorganisms, according to several authors, can be caused by 50 to 80% of corrosion damage [6-9].

Microbiological corrosion can proceed independently and accompany electrochemical, soil, atmospheric and other types of corrosion of metals. The action of microorganisms on metals can occur differently. First of all, corrosive metabolites of microorganisms can cause corrosion: mineral and organic acids and bases, enzymes and others. They create a corrosive medium in which corrosion occurs in the presence of water according to the usual laws of electrochemistry. Colonies of microorganisms can create on the surface of metals growths and films of mycelium or mucus, under which ulcerative (pitting) corrosion can develop [10-13].

The problem of protecting building constructions, buildings and structures from aggressive chemical and biological influences of the environment is of great importance. In public buildings and structures, in particular in subways, in areas with high humidity and certain climatic conditions, microbiological corrosion becomes an important factor affecting the reliability and durability of structures made of metal, concrete and reinforced concrete.

To protect reinforced concrete structures from biological corrosion, an effective and basic method is surface treatment with bactericidal agents. These include synthetic biocides, as well as substances containing metal ions that penetrate into the cells inhibit respiratory chain enzymes and disrupt oxidative phosphorylation processes, resulting in the cell killing. In addition, with their action, cytoplasmic proteins can be coagulated [14-16].

The purpose of this work is to study the effect of biocides based on copolymers and copper sulfate on the development of corrosive-dangerous microorganisms.

Materials and methods. The bactericidal properties of copper sulfate and N, N-dimethyl-N, N-diallyl ammonium chloride copolymers (DMDAAC) with N, N-dimethylacrylamide (DMAA) with respect to thiobacteria and SRB, as well as heterotrophic microorganisms have been studied. Copolymers were synthesized and provided by Doctor of Chemistry, associate professor of JSC “Kazakh-British Technical University” K.Zh. Abdiyev.

For cultivation of *Thiobacillusthioparus* bacteria, the Bayerink’s medium were used, *Thiobacillus thiooxidans* – Waxman’s medium, *Thiobacillusferrooxidans* – medium 9K, *Thiobacillusdenitrificans* – Baalsrud’s medium. Sulfate-reducing bacteria (SRB) were grown on Postgate's medium [17]. To study the effect of biocides on the development of these bacteria, they were cultured on appropriate selective media into which the biocides investigated were added in various concentrations: 0.01-1.0%. Their impact was assessed by the presence or absence of growth of microorganisms.

The effect of biocides on the growth of heterotrophic bacteria, fungi and yeast was verified by diffusion into agar from wells on solid nutrient media (nutrient agar, potato-dextrose agar and glucose-peptone agar) at concentrations of 0.01-1%. Their effect was judged by the zones of suppression of the growth of these microorganisms.

Research results and discussion. Thionic and sulfate-reducing bacteria, as well as heterotrophic microorganisms, were isolated from the damaged sites of reinforced concrete structures. The bactericidal properties of copper sulfate and N, N-dimethyl-N, N-diallyl ammonium chloride copolymers (DMDAAC) with N, N-dimethylacrylamide (DMAA) with respect to isolated microflora. Copolymers with different mole composition were used:

- 1) DMDAAC :DMAA – 95:5 (B1)
- 2) DMDAAC :DMAA – 80:20 (B2)
- 3) DMDAAC :DMAA – 75:25 (B3).

The results of the study showed that the development of *Thiobacillusthiooxidans* bacteria was noted at concentrations of synthetic biocides and copper sulfate 0.01-0.5%, higher concentration of 1% inhibited the growth of these bacteria (Table 1).

Table 1 – Influence of the biocides under study on bacterial growth *T. thiooxidans*

Biocides	Concentration of biocides, %				
	0,01	0,05	0,1	0,5	1,0
B1	+	+	+	+	–
B2	+	+	+	+	–
B3	+	+	+	+	–
CuSO ₄	+	+	+	+	–

Note. «+» - marked growth, «-» - growth is absent.

The development of *T. ferrooxidans* was inhibited even at lower concentrations of the test compounds. Thus, under the influence of copolymers B1, B2 and B3, their growth was not observed at concentration of 0.1% (Table 2). When biocide B3 was used at concentration of 0.05%, a slight growth of these bacteria was noted. Less effective was copper sulfate, which inhibited their growth at concentration of 1%.

For bacteria *T. thioparus* and *T. denitrificans*, inhibition of growth occurred at concentration of synthetic compounds of 0.5%. CuSO₄ suppressed the growth of these bacteria at concentration of 1% (Table 3, 4).

Table 2 – Influence of the biocides under study on bacterial growth *T. ferrooxidans*

Biocides	Concentration of biocides, %				
	0,01	0,05	0,1	0,5	1,0
B1	+	+	–	–	–
B2	+	+	–	–	–
B3	+	+	–	–	–
CuSO ₄	+	+	+	+	–

Note. «+» - marked growth, «–» - growth is absent.

Table 3 – Influence of biocides under study on bacterial growth *T. thioparus*

Biocides	Concentration of biocides, %				
	0,01	0,05	0,1	0,5	1,0
B1	+	+	+	–	–
B2	+	+	+	–	–
B3	+	+	+	–	–
CuSO ₄	+	+	+	+	–

Note. «+» - marked growth, «–» - growth is absent.

Table 4 – Influence of biocides under study on bacterial growth *T. denitrificans*

Biocides	Concentration of biocides, %				
	0,01	0,05	0,1	0,5	1,0
B1	+	+	+	–	–
B2	+	+	+	–	–
B3	+	+	+	–	–
CuSO ₄	+	+	+	+	–

Note. «+» - marked growth, «–» - growth is absent.

SRB reacted to the addition of copolymers of DMDAAC and DMAA starting at concentration of 0.1%. Copper sulfate inhibited the growth of bacteria, starting at concentration of 0.5% (Table 5).

Table 5 – Effect of biocides under study on the growth of sulfate-reducing bacteria

Biocides	Concentration of biocides, %				
	0,01	0,05	0,1	0,5	1,0
B1	+	+	–	–	–
B2	+	+	–	–	–
B3	+	+	–	–	–
CuSO ₄	+	+	+	–	–

Note. «+» - marked growth, «–» - growth is absent.

The study of the effect of biocides on the heterotrophic microflora showed that at concentration of 0.01-0.5% they did not exert an inhibitory effect on bacteria and fungi. Their effect on the growth of microorganisms occurred at concentration of 1% (Table 6, 7).

Table 6 – Influence of biocides under study on the growth of heterotrophic bacteria

Culture	Zones of growth suppression under the action of biocides, mm			
	B1	B2	B3	CuSO ₄
A2-1	14,7±0,6	14,3±0,6	–	12,7±0,6
A2-2	14,3±0,6	14,7±0,6	–	14,3±0,6
A2-4	15,3±1,5	15,3±0,6	–	15,3±2,1
A2-6	14,0±1,0	15,7±1,2	–	16,0±1,0
A5-1	13,7±0,6	16,0±0,6	11,0±0	14,3±0,6
A5-2	16,0±5,2	17,0±0	11,0±0	7,3±1,2
A5-3	13,7±0,6	17,0±0	11,3±0,6	15,3±0,6
G2-1	15,0±0	16,3±0,6	12,3±0,6	17,3±0,6
G2-2	14,7±0,6	16,7±0,6	13,3±1,5	18,0±1,0
G2-3	7,7±0,6	17,0±1,0	11,3±0,6	16,7±0,6
G2-6	14,7±0,6	15,7±0,6	–	16,0±1,0
G2-7	15,3±1,5	14,7±0,6	–	14,0±1,0
G5-1	13,7±0,6	15,7±0,6	11,0±0	17,7±0,6
G5-2	13,7±0,6	14,3±0,6	–	13,3±1,2

Note. Significance level $p < 0,05$.

It can be seen from the table that under the influence of biocide B1, the areas of inhibition of bacterial growth were 7.7-16.0 mm. The G2-3 culture was less sensitive to this compound. Biocide B2 also affected all the bacterial cultures under study, while the clarification zones were 14.3-17.0 mm. B3 was less effective among synthetic biocides, using which growth inhibition zones were observed only in seven cultures and amounted to 11.0-13.3 mm.

All investigated bacteria reacted on CuSO₄. The largest zone of enlightenment was noted in the G2-2 culture, which was 18 mm. The culture A5-2 was less sensitive to the action of copper sulfate (7.3 mm).

Virtually the same effect studied biocides had on fungi. Under the influence of biocides B1 and B2, the areas of suppression of fungal growth were 14.3-18.3 mm. The culture *Penicilliumchrysogenum* 17GM was the most sensitive to these compounds. The growth of this culture was also suppressed by biocide B3, whereas it did not exert a depressing effect on other cultures of fungi. The culture *Aspergillus* sp. 1GM was resistant to the influence of copolymers.

Table 7 – Influence of biocides under investigation on the growth of mycelial fungi

Culture	Zones of growth suppression under the action of biocides, mm			
	B1	B2	B3	CuSO ₄
1GM	–	–	–	21,3±1,2
2GM	14,3±0,6	15,7±2,1	–	22,7±0,6
8GM	15,0±0,1	15,3±2,5	–	15,0±1,7
10GM	14,6±2,1	14,7±1,2	–	16,3±1,5
11GM	14,3±0,6	16,1±1,5	–	14,5±0,6
14GM	15,7±2,1	16,4±0,6	–	27,0±1,7
17GM	18,3±2,5	17,6±1,2	8,3±1,5	26,7±2,9

Note. Significance level $p < 0,05$.

1% solution of copper sulfate suppressed the growth of all fungi studied. The largest enlightenment zones were recorded for 14GM and 17GM cultures, which were 27 mm and 26.7 mm, respectively. The strains 11GM, 8GM and 10GM were less sensitive to the action of copper sulfate.

Yeasts were more sensitive to the action of the test compounds. Thus, the biocides B1 and B2 had a depressing effect on them already at a concentration of 0.1% (Table 8). With increasing concentration the zone of bleaching on solid nutrient media increased. The culture *Exophiala sp. 6gA* was more stable.

Table 8 – Effect of biocides under study on yeast growth

Biocides	Concentration, %	Zones of growth suppression, mm		
		1gA	5gA	6gA
B1	0,1	11,3±0,6	10,7±0,6	-
	0,5	15,3±1,5	15,7±1,2	11,7±1,5
	1,0	16,0±0	16,0±1,0	13,3±0,6
B2	0,1	13,3±0,6	11,7±0,6	10,7±1,2
	0,5	15,3±0,6	12,3±2,1	11,0±1,0
	1,0	16,7±0,6	17,3±2,1	14,0±3,5
B3	0,1	-	-	-
	0,5	-	-	-
	1,0	-	-	-
CuSO ₄	0,1	-	-	-
	0,5	8,3±1,2	10,7±2,5	-
	1,0	31,7±1,5	23,0±2,6	19,7±1,5
<i>Note.</i> Significance level $p < 0,05$.				

Copolymer B3 did not suppress the development of the yeast cultures studied. Copper sulfate began to affect the growth of yeast 1gA and 5gA at a concentration of 0.5%. An increase in the concentration of up to 1% led to a significant increase in the bleaching zones. The strain *Erythrobasidium clade sp. 1gA* was more sensitive.

Conclusions. Thus, the conducted studies showed that copper sulfate possessed the lowest bactericidal action with respect to thionic and sulfate-reducing bacteria. When it was added, the growth of the studied bacteria was suppressed at a concentration of 1%, and only for SRB, their development was not observed at a lower concentration of 0.5%. The copolymers of N, N-dimethyl-N, N-diallyl ammonium chloride (DMDAAC) and N, N-dimethylacrylamide (DMAA) with different mole composition exhibited a more active effect. Their effect on the inhibition of thionic and sulfate-reducing bacteria occurred at concentrations of 0.1-0.5%. *T. ferrooxidans* and SRB were the most sensitive to their effects.

On heterotrophic bacteria and fungi, the compounds tested had a bactericidal effect only at a concentration of 1%. Biocides B1 and B2 had approximately the same activity, while copolymer B3 suppressed the growth of only 7 bacterial cultures and 1 fungus culture. Copolymers B1 and B2 inhibited the development of yeast starting at a concentration of 0.1%. Biocide B3 did not exert a suppressive effect on all yeasts. Copper sulfate was more effective with respect to fungi and yeast, while inhibition of their growth occurred at concentrations of 1% and 0.5%, respectively.

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КОРРОЗИЯЛЫҚ-ҚАУІПТІ МИКРОФЛОРАНЫҢ ӨСП-ӨНУІ МЕН ДАМУЫНА БИОЦИДТЕРДІҢ ӘСЕРІ

Аннотация. Қазіргі таңда ғимараттар мен құрылыстарды қоршаған ортаның агрессивті химиялық және биологиялық әсерінен қорғау мәселесі өте өзекті болып келеді. Микробиологиялық коррозия темірбетон конструкцияларының сенімділігі мен ұзақ мерзімділігіне ықпал ететін маңызды фактор болып табылады. Осыған байланысты, құрылыстар конструкциясы мен ғимараттарды агрессивті биологиялық әсерден қорғау мәселесі өте өзекті. Зерттеудің мақсаты коррозиялық-қауіпті микроорганизмдердің дамуына сополимерлер мен мыс сульфаттарының негізіндегі биоцидтердің әсерін зерттеу болып табылады. Мыс сульфаты тионды және сульфатредуцирлеуші бактерияларды (СРБ) азайтуға қарсы ең аз тиімді ингибиторлық әрекетке ие екендігі анықталды. 1% концентрация қосылған кезде зерттелген бактериялардың өсуі баяулады. Әртүрлі молярлық құрамды сополимерлер анағұрлым белсенділік көрсетті. Олардың СРБ және тионды бактерияларды баяулату әсері 0,1-0,5% концентрациясында орын алды. Олардың әсеріне ең сезімтал *T. ferrooxidans* және СРБ болды. Гетеротрофты бактериялар мен жіпшумақты саңырауқұлақтарда сыналған қосылыстар тек 1% концентрацияда бактерицидтік әсерге ие болды. Мыс сульфаты микромицеттер мен ашытқыларға қатысты тиімді болғанымен, олардың өсуінің баяулауы тиісінше 1% және 0,5% концентрация кезінде байқалды.

Түйін сөздер: биокоррозия, коррозиялық-қауіпті микроорганизмдер, тионды және сульфатредуцирлеуші бактериялар, гетеротрофты микроорганизмдер, биоцидтер.

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ВЛИЯНИЕ БИОЦИДОВ НА РОСТ И РАЗВИТИЕ КОРРОЗИОННО-ОПАСНОЙ МИКРОФЛОРЫ

Аннотация. Проблема защиты зданий и сооружений от агрессивных химических и биологических воздействий окружающей среды в настоящее время становится весьма актуальной. Микробиологическая коррозия является важным фактором, влияющим на надежность и долговечность железобетонных конструкций. В связи с этим большую актуальность представляет проблема защиты строительных конструкций и сооружений от агрессивных биологических воздействий. Целью исследований было изучение влияния биоцидов на основе сополимеров и медного купороса на развитие коррозионно-опасных микроорганизмов. Установлено, что наименее эффективным ингибирующим действием по отношению к тионовым и сульфатредуцирующим бактериям (СРБ) обладал медный купорос. При его добавлении рост исследуемых бактерий подавлялся при концентрации 1%. Более активное действие проявляли сополимеры с разным мольным составом. Их воздействие по ингибированию тионовых бактерий и СРБ происходило при концентрациях 0,1-0,5%. Самыми чувствительными к их воздействию были *T. ferrooxidans* и СРБ. На гетеротрофные бактерии и мицелиальные грибы исследуемые соединения оказывали бактерицидное действие только при концентрации 1%. Медный купорос был более эффективен по отношению к микромицетам и дрожжам, при этом ингибирование их роста происходило при концентрациях 1% и 0,5% соответственно.

Ключевые слова: биокоррозия, коррозионно-опасные микроорганизмы, тионовые и сульфатредуцирующие бактерии, гетеротрофные микроорганизмы, биоциды.

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