The effect of triazoloazepine bromide with biocidal activity on microbial copper corrosion

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Abstract

The study deals with the effect of triazoloazepine bromide with biocidal activity against sulfate-reducing bacteria on microbial copper corrosion in neutral water–salt Postgate "B" medium with the enrichment culture of bacteria, isolated from the biofilm, formed on the metal surfaces of sewage treatment facilities. It has been established that the studied substance also inhibits the growth of iron-reducing bacteria, denitrifying bacteria and ammonifying bacteria, which ensure the stable growth of the most numerous and aggressive constituent of the enrichment culture – sulfate-reducing bacteria. It has been demonstrated that triazoloazepine bromide increases the redox potential of the corrosive medium by up to 170 mV. This enables the change of enzymatic reactions direction and considerable inhibition of sulfate-reducing activity of the enrichment culture bacteria. The morphology of the copper coupons surface after the exposure to the corrosive medium with bacterial sulfate-reduction has been examined. It has been defined that without triazoloazepine bromide the biofilm is a chaotic accumulation of bacteria in the polymeric matrix. In the presence of triazoloazepine bromide, only individual bacteria can be observed in the corrosive medium on the surface of the biofilm, which has a loose structure. It has been estimated that triazoloazepine bromide decreases the rate of microbial copper corrosion by a factor of 11.7.

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1. Introduction

Considerable loss of metal fund to microbial corrosion and man-caused risks demand the search of effective inhibitors with biocidal activity and the establishment of their action mechanism. Most of the researches are aimed at studying steel biocorrosion under the influence of sulfate-reducing bacteria (SRB) and its inhibition [1–4]. Under anaerobic

conditions that facilitate the growth of SRB, technological copper equipment is also subject to microbial corrosion [5]. It has been established [6, 7] that SRB are able to colonize the copper surface and create a biofilm. SRB hydrogenases can accept electrons from the copper surface and ensure the reduction of sulfate ions, which leads to the production of biogenic hydrogen sulfide [6]. Under biocorrosion sulfides, most probably chalcocite sulfides (Cu_2S), are formed on the copper surface [7]. The biofilm heterogeneity and the sulfides formed create local differences on the metal surface and thus accelerate corrosive processes [4]. Considerable attention is paid to the study of the biofilm and the effect of inhibitors–biocides on its formation [8, 9].

Triazoloazepine derivatives, which demonstrate biocidal activity against sulfate-reducing bacteria and act as effective inhibitors of microbial steel corrosion [10], can be promising for the inhibition of microbial copper corrosion.

In the study of microbial corrosion it is considered effective to use the enrichment cultures of sulfate-reducing bacteria isolated from natural or man-made objects, because such cultures contain bacteria-satellites, which make the enrichment culture more resistant to external factors, including the activity of inhibitors–biocides [11, 12].

The aim of this paper is to study the effect of triazoloazepine bromide with biocidal activity on the microbial copper corrosion in a neutral water–salt medium in the presence of an enrichment culture of sulfate-reducing bacteria.

2. Experimental

2.1. Chemicals and organisms

(1-[2-(4-Bromophenyl)-2-oxoethyl]-3-[4-methoxyphenylamino)methyl]-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepin-1-ium bromide (TAB) [13] was studied as an inhibitor–biocide. The enrichment culture of SRB was gathered from metal equipment surfaces of sewage treatment constructions (Chernihiv) [14]. It consisted of: sulfate-reducing bacteria ($2.5\cdot10^9$ cell·ml^{-1}), iron-reducing bacteria ($1.3\cdot10^3$ cell·ml^{-1}), denitrifying bacteria ($6.0\cdot10^1$ cell·ml^{-1}) and ammonifying bacteria ($7.0\cdot10^6$ cell·ml^{-1}). The number of microorganisms was calculated by using the method of decimal serial dilution during the bacteria seeding to the correspondent liquid selective media [15]. Iron-reducing bacteria, denitrifying bacteria and ammonifying bacteria are natural satellites of sulfate-reducing bacteria.

2.2. Gravimetric method

The corrosion tests were performed using the gravimetric method. Copper coupons (99.9% Cu) polished to the 4–5 class of accuracy were used. The surface area of the coupons was 3.6×10^{-4} m^2. They were immersed in sealed glass bottles about 120 ml, filled with Postgate "B" model medium [16] with the enrichment culture of sulfate-reducing bacteria, with and without 0.5 g/l of triazoloazepine bromide (TAB). The exposure time was up to

15 days and the temperature was 300 K (optimal temperature for the development of sulfate-reducing bacteria).

The Postgate "B" medium composition is as follows, per liter: KH_2PO_4 0.5 g; NH_4Cl 1.0 g; $CaSO_4 \cdot 2H_2O$ 1.0 g; $MgSO_4$ 2.0 g; calcium lactate 3.5 g; yeast extract (5%) 10 ml; $FeSO_4 \cdot 7H_2O$ (5% solution in 1% HCl) 10 ml; ascorbic acid (5%) 2 ml; $NaHCO_3$ (5%) to reach pH 7.5. The acidity was measured with a pH/ION 340i pH-meter/ionomer. The Postgate "B" medium was chosen as it is optimal for the development of sulfate-reducing bacteria and does not suppress the growth of their satellites.

The corrosion rates with or without the inhibitors were calculated using the formula:

$$k = \Delta m/(S \cdot \tau),$$

where Δm is the sample weight loss in g, S is the area in m^2, and τ is the exposure time in hours.

The corrosion inhibition coefficient was calculated using the formula:

$$\gamma = k / k',$$

where k and k' are the corrosion rates without and with the inhibitor, respectively.

The inhibition efficiency (IE%) was calculated using the following equation:

$$IE\% = (1 - 1/\gamma) \times 100\%.$$

2.3. Microbiology method

The biocidal activity of triazoloazepine bromide against the components of the enrichment culture was defined by the agar diffusion method and measured according to the diameter of bacteria growth inhibition zone in the corresponding agar media: iron-reducing bacteria (IRB) were seeded to the Kalinenko medium, denitrifying bacteria (DNB) to the Giltay medium, and ammonifying bacteria (AMB) to meat peptone broth [15].

2.4. Analytical methods

The TAB effect on the microbial copper corrosion was estimated by the change in redox potential and the concentration of biogenic hydrogen sulfide. The redox potential (E, mV) of the corrosive medium was defined using the oxidation–reduction potential sensor and a LabQuest 2 recording device (Vernier Software & Technology). A silver/silver chloride comparison electrode was used. The Measurement error was ± 0.5 mV.

The concentration of biogenic hydrogen sulfide was measured by iodometric titration [17].

2.5. Inversion voltammetry

After immersing copper coupons in Postgate "B" medium, the medium was tested using the voltammetry method to identify copper solubility in it. In order to transfer copper ions into the solution in electrochemically active forms, a PDP-Lab programmable two-chamber

furnace (Research and Development Enterprise "Tomanalit", RF) was used. The samples (10 ml) were prepared by a method that combines hydro mineralization (conc. HNO_3, 30% H_2O_2) and dry ashing that was repeated two or three times. Ash was dissolved in 0.2 ml of conc. formic acid and diluted to 10 ml by double distilled water. Distilled water (10 ml), conc. formic acid (0.2 ml) and a sample aliquot (20 μl) were added to the quartz electrochemical chamber.

The content of Cu^{2+} ions was determined on a TA-Lab voltammetry analyzer (Research and Development Enterprise "Tomanalit" RF) in a three-electrode electrochemical cell. An amalgam electrode was used as the indicator electrode. Two AgCl electrodes filled with a 1 M potassium chloride solution were used as the reference electrode and the auxiliary electrode, respectively.

The analysis was performed in the background electrolyte that contains 200 μl of concentrated formic acid ("chemically pure" grade) under the following conditions: electrochemical cleaning of the indicator electrode at a potential of +0.050 V for 10 s, followed by metal accumulation on the surface of the indicator electrode at –1.000 V for 10 s, solution calming at –0.400 V for 5 s, and anodic oxidation at a linear potential sweep rate of 80 $mV \cdot s^{-1}$.

A sample of each solution was analyzed in three parallel experiments. Determination of copper ions was performed by the additives method using a standard solution that contained 1 $mg \cdot l^{-1}$ of Cu^{2+}. The solution was prepared from samples standardized by a state institution (1.0 mg/cm^3) and double distilled water. The copper concentration was calculated by the one addition method provided by the manufacturer of the TA-Lab device and implemented in a specialized computer program, version 3.6.10.

2.6. Scanning Electron Microscopy (SEM)

The surface analysis of the biofilm formed on the surface of metal samples was performed with a scanning electron microscope (SEM). To fix the grown biofilm to the steel surface, the coupons were immersed for 1 h in a 2% glutaraldehyde solution, dehydrated with 4 ethanol solutions (15 min in each) with volume concentrations of 25%, 50%, 75% and 100% successively, and air dried overnight [18]. After the fixation, the coupons were examined using a FEIE-SEM XL 30 field emission scanning electron microscope. The electron microscopic pictures were taken in the secondary electrons mode. The maximum residual pressure in the microscope column was no more than $6.7 \cdot 10^{-4}$ Pa at the gun current of 76 mA.

2.7. Quantum chemical calculations

Calculations of the electric charges on the molecule atoms and simulation conformation of the molecule were done using the Hyperchem 7.0. software (Hypercube, Inc.). The geometry was optimized using the Fletcher–Reeves conjugate gradient method with PM3

parameterization. Considering the dissociation of salts in the water–salt medium, effective charges were calculated for triazoloazepinium cations.

Energy characteristics such as the energy of the highest occupied molecular orbital (E_{HOMO}, eV), the energy of the lowest unoccupied molecular orbital (E_{LUMO}, eV), and the energy gap ($\Delta E = E_{HOMO} - E_{LUMO}$) have also been calculated [19].

3. Results and discussion

The triazoloazepinium bromide studied demonstrates the high antimicrobial activity against sulfate-reducing bacteria that has been shown in our previous studies [10]. It has also been found that TAB inhibits the growth of iron-reducing bacteria, denitrifying bacteria and ammonifying bacteria (Figure 1). This antimicrobial activity of TAB is important because the mentioned bacteria provide conditions for the stable growth of the most numerous and most aggressive component of the enrichment culture – sulfate-reducing bacteria.

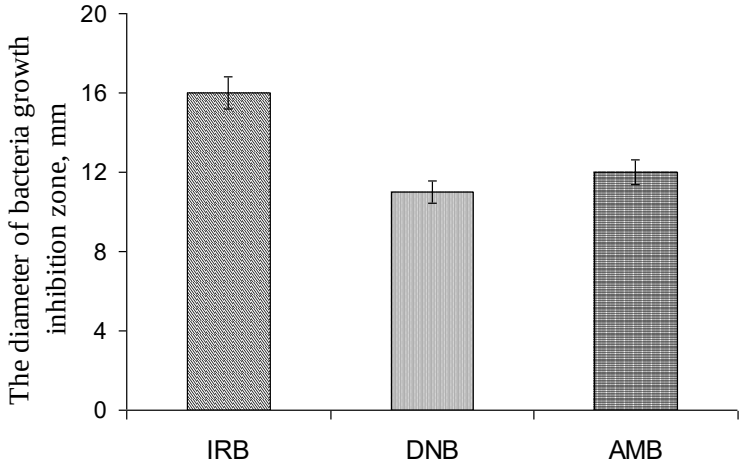

Figure 1. Antimicrobial properties of triazoloazepinium bromide (2% solution in ethyl alcohol) against the components of microbial community.

In the course of copper biocorrosion the composition of Postgate “B” medium, which is a multicomponent system, constantly changes. This is primarily due to the formation of different metabolic products of the enrichment culture bacteria. The main metabolite of SRB and their satellites is hydrogen sulfide, with the possible formation of acetate ions, formate ions, *etc*. [19, 20]. The redox potential is an important thermodynamic and kinetic factor for such a system [21]. Its change in time under microbial copper corrosion with and without triazoloazepinium bromide is demonstrated in Figure 2a. In the corrosive medium without the biocide, on the second day of copper coupon exposure the potential value reaches its minimum, which indicates the accumulation of products with reducing properties. This is consistent with the rapid increase in biogenic hydrogen sulfide concentration (Figure 2b). After the lag-phase, the duration of which can be estimated as

1 day for the bacteria culture studied, the active growth of microorganisms starts, which lasts until the 5th day. Stabilization of the redox potential value indicates the end of the active bacteria growth phase. The cessation of bacteria growth is to some extent facilitated by the high concentration of hydrogen sulfide (about 290–330 mg/l), which decreases after the 6th day, probably due to the formation of sulfides.

The presence of TAB in the Postgate "B" medium inoculated with the bacteria enrichment culture increases the initial value of *E* by 30 mV. This enables the direction change of certain enzymatic reactions, which are highly specific. The comparison of redox potential values shows that within all the experiment *E* is 30–170 mV more positive in the medium with triazoloazepinium bromide compared to the medium without the inhibitor biocide (Figure 2a). At the same time, within the first day a significant shift of *E* towards the positive values can be observed. On the second day, the redox potential grows insignificantly and remains within –240 to –250 mV until the end of the experiment. This value differs from the optimal value for the growth of SRB [22] and can lead to the death of the microbial cells.

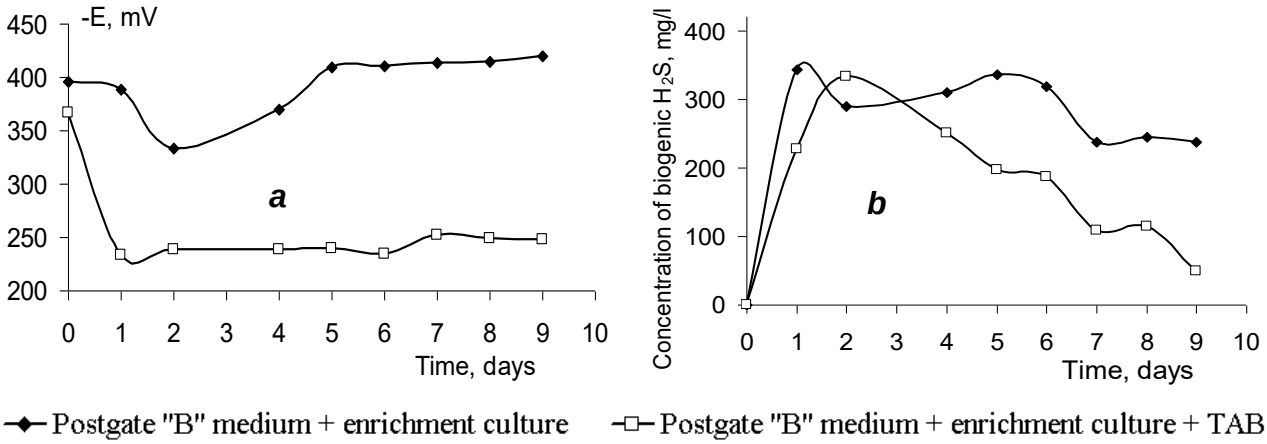

Figure 2. The dynamics of the redox potential of the corrosive medium (a) and the concentration of hydrogen sulfide (b) at microbial copper corrosion.

The effect of the biocide leads to an increase in hydrogen sulfide concentration on the second day to 320 mg/l. This can be explained as a reaction of the microorganisms on its activity. Further the content of H_2S in the medium decreases (Figure 2b), indicating the considerable inhibition of bacteria sulfate-reducing activity by the biocide.

A visual inspection showed that after 10 days of testing, the corrosive medium in the tube with TAB was transparent. This indicates either a comparatively low titre of the microorganisms or their full destruction. Copper coupons after the tests were shiny, without visible sulfide film. The medium in the tube without the biocide was of black color and copper coupons were covered with a dense sulfide film.

Analysis of the corrosive medium showed that the concentration of Cu^{2+} ions in the medium without triazoloazepinium bromide after 10 days of copper coupons exposure

equaled 0.045 ± 0.007 mg/l, *versus* 0.110 ± 0.020 mg/l in the solution with TAB (Figure 3). The lower (2.4-fold) concentration of Cu(II) ions in the solution without the inhibitor is explained by their active bonding with the formation of sulfides on the surface of copper coupons. As it was mentioned earlier, these corrosion products were visible. The formation of sulfides explains the decrease in the hydrogen sulfide concentration in the corrosive medium, which was recorded on the 6th day of exposure, and the decrease in the concentration of Cu^{2+} ions in the corrosive medium.

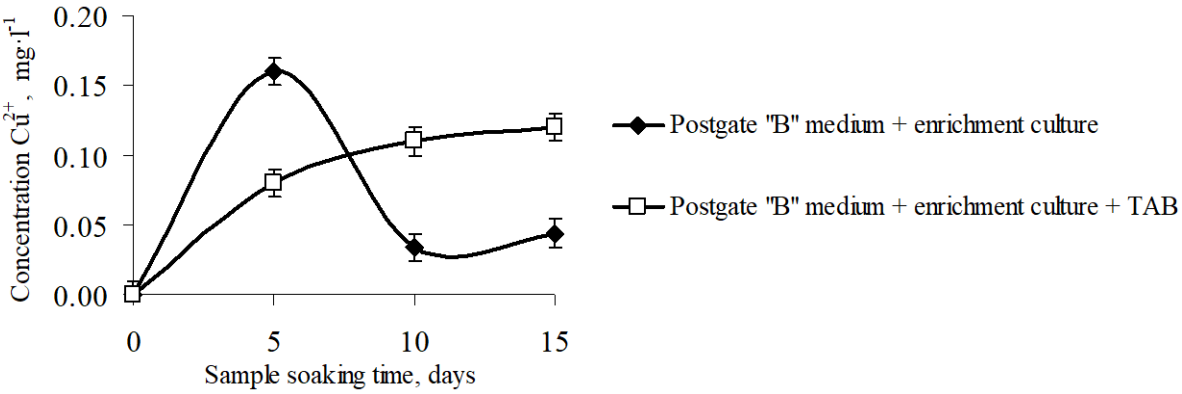

Figure 3. The correlation between the concentration of Cu^{2+} ions in Postgate “B” medium and the copper coupons exposure time.

The morphology of the copper coupons surface after immersing in the corrosive medium with bacterial sulfate reduction is demonstrated in Figure 4. The photos indicate that the studied bacteria enrichment culture forms a biofilm on the copper surface. It looks as chaotic accumulation of bacteria in the polymer matrix (Figure 4a).

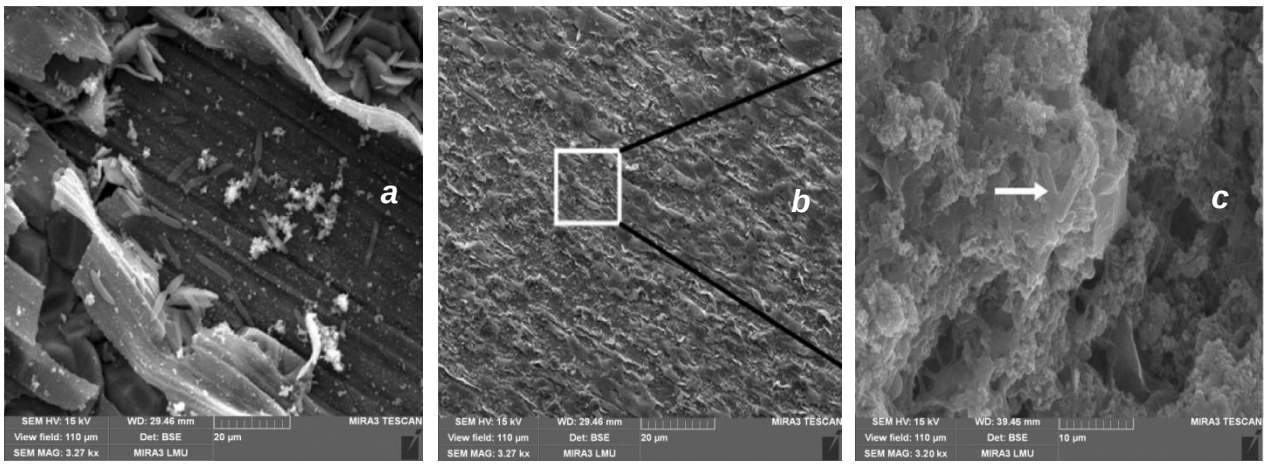

Figure 4. FE-SEM images of the biofilm formed on copper surface after exposure to Postgate “B” medium with enrichment culture of sulfate-reducing bacteria: a) without TAB (×10 000); b) in the presence of TAB (×200); c) in the presence of TAB (×10 000).

In such biofilm there are active processes of microbial corrosion. The copper corrosion rate calculated using the gravimetric method equals 6.7 mg/(m^2·h). This value is slightly lower than the copper corrosion rate in the presence of *Desulfovibrio vulgaris* estimated in [7], and is close to the corrosion rate of copper inoculated by the SRB culture isolated from the ferrosphere of a corroded gas pipeline [23]. The morphology of copper coupons surface after immersing in corrosive medium with the biocide differs substantially (Figure 4b). On the surface of the biofilm, which has a loose structure, only individual bacteria are observed (Figure 4c). Such biofilm is not corrosively aggressive. The microbial copper corrosion rate decreases by a factor of 11.7, which determines the inhibition efficiency of 93.5%.

It can be assumed that in the test with the biocide, the biofilm is formed on the layer of adsorbed TAB. The ability of TAB to be adsorbed on copper is caused by the presence of negatively charged atoms in its molecule (nitrogen atoms of the heterocyclic system and amide group, oxygen atoms of the carbonyl and methoxy groups), which are able to transfer electrons to the unoccupied metal orbitals (Figure 5a), and the molecule conformation (Figure 5b), which contributes to the interaction of nitrogen atoms with the metal surface. What is more, according to the quantum-chemical calculations, the studied triazoloazepinium bromide is characterized by a sufficient activity, the value of the energy gap being ΔE = 5.417 eV.

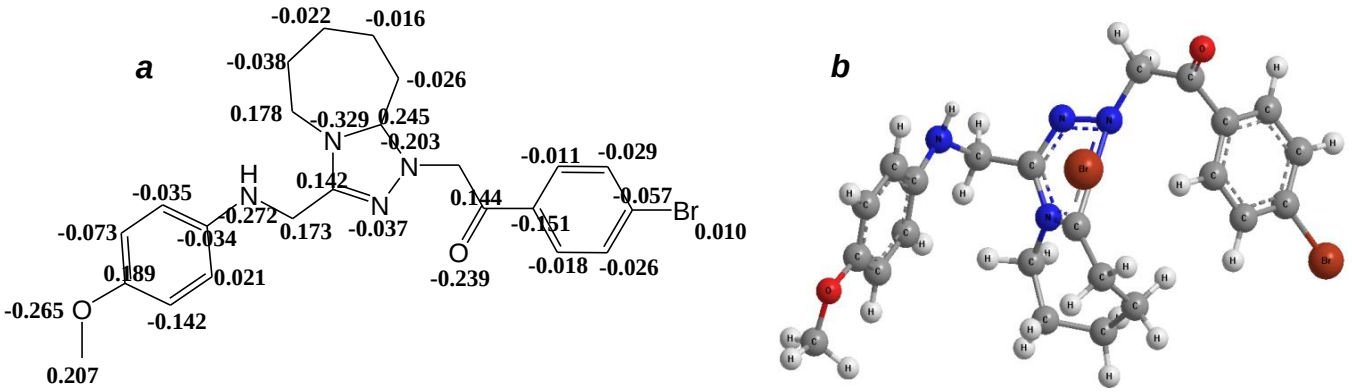

Figure 5. The distribution of charges in the atoms of triazoloazepinium cation (a) and molecule conformation (b).

Conclusions

1. Triazoloazepinium bromide with biocidal activity against the components of the enrichment culture increases the redox potential of the corrosive medium by up to 170 mV, which leads to a significant inhibition of bacteria sulfate reducing activity.
2. In the presence of triazoloazepinium bromide in the water–salt medium with bacterial sulfate reduction the rate of microbial copper corrosion decreases by a factor of 11.7.

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