

INVESTIGATION OF THE BACTERICIDAL-INHIBITORY PROPERTY OF THE REAGENT IN LABORATORY CONDITIONS

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The corrosion protection capability of reagent C-1 with bactericidal-inhibitory properties was studied in laboratory conditions in neutral, acidic and alkaline mediums and the concentration of the reagent was in the range of 10-25 mg/l. Desulfomicrobium and desulfovibrio desulfuricans strains of sulphate-reducing bacteria extracted from formation water of oil well no. 1802 of Bibiheybatneft OGPD, SOCAR field were used as the research object. For comparison, reagent free and reagent-based mediums were used in the research process. In laboratory conditions, the effect of reagent C-1 on the incubation period of sulfate-reducing bacteria was investigated for fifteen days in postgate-“B” nutrient medium. It was determined that having a bactericidal property, reagent C-1 affects the life activity of bacteria significantly. It was revealed that the highest bactericidal effect occurs at a concentration of 25 mg/l of reagent C-1 and the effect of corrosion protection from bacteria is 85%.

Keywords: bactericide, corrosion, protection effect, hydrogen sulphide, sulfate-reducing bacteria, neutral medium, acidic medium, alkaline medium

Received 22. 11. 2023, Accepted 18. 12. 2023

1. Introduction

One of the most serious and global problems of developed industrial countries is corrosion. Taking into account that corrosion is such a large-scale problem, various ways of its prevention are being prospected. Due to the great importance of corrosion protection of metals used in the petrochemical industry, the research of the corrosion process and obtaining of anti-corrosion organic compounds remain one of the important issues to be solved. As a result of accidents occurring during corrosion, the damage to the nature, including the living world is quite large due to the release of toxic chemicals and oil products into the medium.

It is known that the presence of pH-changing ions in the oil and formation water, as well as the formation of gases dissolved in the formation water, make the environment aggressively corrosive, which increases the speed of both electrochemical and chemical corrosion. Moreover, participation of sulfate-reducing bacteria (SRB) in the process increases the rate of electrochemical corrosion of the facilities while intensifying the process. As a result, the oil industry is harmed both economically and environmentally. Despite the fact that the process of corrosion of metals in hydrogen sulphide mediums has been widely studied by researchers, namely finding ways to solve this problem in the oil industry is currently of both practical and economic importance.

The presence of hydrogen sulphide in oil and formation water causes intensive corrosion of underground facilities in oil wells, the inner surface of oil pipelines, as well as oil storage and deposit tanks, and facilities being operated in an oil refinery. For this reason, the issue of selecting, testing and wide application of chemical reagents for weakening and stopping the corrosion process

with the presence of hydrogen sulphide has always been relevant. [1-4].

Currently, corrosion, which is considered a global problem, remains a very serious problem in Azerbaijan Republic, along with developed countries with oil industry, in Oil and Gas, Petrochemical industry and other fields. In recent years, the corrosion process occurred by the effect of high temperature and pressure in aggressive mediums in industry poses a serious threat in industrialized countries with high metal reserves. Making equipment from corrosion-resistant materials cannot always ensure their longevity and reliability. In this regard, it is necessary to use other methods of corrosion protection. Among the methods used to combat corrosion on a large scale, the method of protection through inhibitors is the simplest, most effective, and in many cases is economically advantageous. However, since the variety of environments and conditions under which the facilities are used (temperature, pressure, etc.) differ sharply, the proposed reagents do not show a high effect in any condition. Therefore, reagents suitable for each condition should be designed. On the other hand, currently none of the five Caspian countries with developed oil and gas extraction and oil and gas processing industries have inhibitor production that will supply the country. Therefore, the creation of multifunctional corrosion inhibitors with a wide range of raw materials remains an urgent problem.

It should be noted that since Azerbaijan has developed oil and gas extraction, oil and gas processing, petrochemical and chemical industries, corrosion of metal units and facilities used in these industries is one of the most serious problems for our country. Depending on the environment, in addition to the corrosion process, other processes occur, which in turn complicates the solution of the problem. This is more noticeable in the oil indus-

try. Thus, corrosion occurs rapidly in oil extraction, storage and transportation systems, as well as during the processes with the participation of microorganisms and salt deposit. In high concentrations of hydrogen sulphide and carbon dioxide, as well as in high amounts of sulfate-reducing bacteria, the creation of highly effective inhibitors that can protect oil industry units and facilities from hydrogen sulphide, carbon dioxide corrosion, and also from biological corrosion remains an urgent problem in our country and in the world. To solve this type of problem, such multifunctional systems should be created that can neutralize all three above-mentioned corrosive environments. Of course, the creation of such reagents creates certain difficulties, but it is considered expedient to continue research in this field [5-10].

The work objective is to investigate bactericidal-inhibitory properties of a new reagent of organic origin in laboratory conditions.

2. Experimental part

Steel samples of Ct3 brand were used to investigate the corrosion intensity (table 1).

Table 1 Chemical composition of Ct3 brand steel (%)

Element	C	Mn	Si	P	S	Cr	Ni	Cu	Fe
Content (% wt.)	0.2	0.5	0.15	0.04	0.05	0.30	0.20	0.20	98.36

The chemical composition of the reagent of organic origin, the inhibitory-bactericidal properties of which are studied in laboratory conditions, is C₁₁H₉Cl₂O (conditional name C-1) [12].

In order to determine the corrosion rate of steel plates of Ct3 brand with dimensions of 30x20x1 mm according to the mass loss, studies were conducted in laboratory conditions for 6 hours at a temperature of 25 °C at concentrations of 10; 15; 20 and 25 mg/l of reagent C-1.

Plates made of Ct3 brand steel were polished on a grinding balance, they were weighed on an analytical weighing machine after having been cleaned with acetone and alcohol. For comparison, the experiments were carried out both without reagent and in parallel with the reagent under the same conditions.

Table 2. Chemical composition of Postgate “B” nutrient medium

Component	NH ₄ Cl	K ₂ PO ₄	MgSO ₄ x7H ₂ O	CaSO ₄	Lactate-Ca	Na ₂ S	Na ₂ SO ₃	FeSO ₄ (1% in HCl 5% solution)
Concentration (g.l ⁻¹)	1.0	0.5	2.0	1.0	2.6	0.2	2.0	0.5

The reagent required for the composition of the nutrient medium is calculated for one liter of water, and the pH of the medium should be in the range of 7-7.5. The pH of the medium is checked with universal indicator paper. Also, the growth of sulfate-reducing bacteria in Postgate-“B” nutrient medium is optimized by introducing special additives. Researches were conducted in static

After the laboratory experiment was completed, the steel plates were removed from the environment and cleaned of corrosion products on the surface. For this, the plates were cleaned with cotton in a solution made of 10% hydrochloric acid and 40% formalin, washed in running water and dried in acetone. The plates were kept in a desiccator for 10-12 hours to bring them to a constant weight both before and after the experiment. The plates were then weighed on the weighing machine again.

The corrosion rate was calculated according to the following mathematical expression.

$$K = \frac{m_1 - m_2}{S \cdot \tau} \tag{1}$$

where, m₁ is pre-experiment weight of the sample; m₂ is the weight of the sample after removing the corrosion product, gr; S is the area of the sample, m²; τ - is the duration of the experiment, hour.

Mathematical expression used to calculate the deceleration factor

$$\gamma = \frac{K_0}{K_{inh}} \tag{2}$$

where, K₀ is reagent free, K_{inh.} is corrosion rate with reagent (g/m²·hour).

The protection effect of reagent C-1 was calculated by the following formula

$$Z = \frac{K_0 - K}{K_0} 100\% \tag{3}$$

where, K₀ is reagent free, K_{inh.} is corrosion rate with reagent (g/m²·hour).

The penetration depth is determined as follows in accordance with the corrosion rate.

$$\Pi_k = 1,12K \tag{4}$$

In the research process, Postgate -“B” nutrient medium was used for the growth and maturation of sulfate-reducing bacteria. The appearance of bacteria under a microscope was studied through the microscope of MBI-6. A limited dilution method was used to determine the quantity of living tissues. In such a nutritious medium, which is considered more suitable for the growth of SRB, they have the ability to reproduce intensively (table 2.) [11].

conditions. All corrosion studies were conducted under thermostatic conditions to ensure optimal development performance of SRB.

In order to determine the bactericidal properties of reagent C-1 in laboratory conditions, *Desulfomicrobium* and *Desulfovibrio desulfuricans* strains of sulfate-reduc-

ing bacteria were used during the experiment. Sulfate-reducing bacteria used for the experiment were taken from formation waters of well No. 1082 of Bibiheybatneft OGPD field, SOCAR.

The facilities used in the experiment; exsiccator, microscope MBI-1, MBR-1, autoclave AQ-1, AV-1, sterilizer, thermostat, medical syringe of 1-2 ml, 0.1, flasks of 0.5 and 1 liter, test bottles, penicillin bottles, rubber stoppers, transmission glasses and item glasses. Corrosion medium, containers and other items should be sterilized in an autoclave condition during research with the participation of sulfate-reducing bacteria. Such a situation prevents the growth of external bacteria in the investigated medium.

To determine the bactericidal properties of the reagent, it was used in pre-sterilized test bottles according to the method [12]. The bactericidal properties of the reagent were investigated mainly by being observed for fifteen days and at the end, based on the calculation of the concentration of hydrogen sulfide formed in Postgate - "B" medium. The formation of hydrogen sulfide in the medium was determined by the iodometric titration method. Both the concentration of biogenic hydrogen sulfide and the number of microorganism cells were determined daily.

To sterilize the nutrient Postgate-"B" medium and remove dissolved molecular oxygen, it was heated to boiling and then rapidly cooled to 35°C.

The solutions of the reagent the amount of which was pre-calculated (10-25mg/l) were added to sterilized test bottles together with Postgate-"B" solution and kept in a thermostat at a temperature of 30-35°C for fifteen days. During the experiment, before determining the formation of hydrogen sulfide by the method of iodimetric titration, it is possible to visually determine the growth of sulfate-reducing bacteria in the nutrient medium according to the formation of a dark-colored deposit at the bottom of the bottles. Sulfate-reducing bacteria are mostly black in colony form, which is due to the biogenic hydrogen sulfide they synthesize as a result of their life activity. When the reagent taken for the research has a bactericidal effect, white deposit occurs at the bottom of the test bottles due to the suspension of metabolism of sulfate-reducing bacteria.

The number of sulfate-reducing bacteria cells in 1 ml of initial suspension is calculated by the following formula:

$$M = \frac{1000an}{hs} \quad (5)$$

where M is the number of cells in 1 ml of suspension
 a is the average number of cells per square grid
 h is the depth of the cell (mm) n_0
 S is the area of square grid (mm²)
 n is the dilution rate of suspension.

The growth factor of sulfate-reducing bacterial cells in the presence of the reagent is calculated by the following expression:

$$N, \% = \frac{100(n_0 - n_{reag})}{n_0} \quad (6)$$

where n_0 is the number of microorganisms in reagent-free medium.

N_{reag} is the number of microorganisms in the medium with the reagent.

According to the amount of hydrogen sulfide, the bactericidal effect of the reagent is calculated by the following formula:

$$S, \% = \frac{C_0 - C_{reag}}{C_{reag}} 100 \quad (7)$$

where, C_0 is the concentration of biogenic hydrogen sulfide in reagent-free medium,

C_{reag} is the concentration of biogenic hydrogen sulfide in the medium with reagent.

The variation coefficient of the concentration of hydrogen sulfide is found by the following mathematical expression:

$$\gamma_c = \frac{C(H_2S)_0}{C(H_2S)_{reag}} \quad (8)$$

where $C(H_2S)_0$ is the concentration of hydrogen sulfide in reagent-free medium,

$C(H_2S)_{reag}$ is the concentration of hydrogen sulfide in the medium with reagent.

3. Results and discussion

In order to determine the corrosion protection effect of reagent C-1, experiments were carried out in a U-shaped unit in laboratory conditions. Researches were carried out in neutral, acidic and alkaline mediums. The results obtained from the research are presented in table 3. As it is seen from table 3, the consumption of the reagent was in the range of 10-25 mg/l. Within this range, the corrosion rate in neutral medium varies between 0.0937-0.0390 g/m².h, the protection effect varies between 88-95%, the corrosion rate in acidic medium varies between 0.1856-0.0265 g/m².h, the protection effect between 86-98%, and the corrosion rate in alkaline medium varies between 0.2048 -0.0614g/m².h, and the protection effect varies between 80-94%. The analysis of the results reveals that the amount of inhibitor used at 25 mg/l is effective for all three mediums. At this time, its corrosion protection efficiency is 94-98%.

As it is seen, reagent C-1 shows the best result (98%) in acidic medium. The reason for the significant decrease in the corrosion rate is due to the property of the reagent to hydrophobe the metal surface. More precisely, the reagent screens the active centers with high energy on the metal surface, isolating them from the aggressive medium and passivates the electrochemical corrosion process.

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Table 3 Protection efficiency of reagent C-1 in different mediums

No.	C _{reag} (mg/l)	K (g/m ² ·hour)	γ (-)	Z (%)
a) neutral medium				
1	0.0	0.7812	-	-
2	10	0.0937	8.34	88
3	15	0.0703	11.11	91
4	20	0.0547	14.28	93
5	25	0.0390	20.03	95
b) acidic environment				
1	0.00	1.3260	-	-
2	10	0.1856	7.14	86
3	15	0.1326	10.00	90
4	20	0.0663	20.00	95
5	25	0.0265	50.03	98
c) alkaline environment				
1	0.00	1.024	-	-
2	10	0.2048	5.00	80
3	15	0.1638	6.25	84
4	20	0.1331	7.69	87
5	25	0.0614	16.67	94

As it is seen from table 3, the consumption of the reagent was in the range of 10-25 mg/l. Within this range, the corrosion rate in neutral medium varies between 0.0937-0.0390 g/m²·h, the protection effect varies between 88-95%, the corrosion rate in acidic medium varies between 0.1856-0.0265 g/m²·h, the protection effect between 86-98%, and the corrosion rate in alkaline medium varies between 0.2048 -0.0614g/m²·h⁻¹, and the protection effect varies between 80-94%. The analysis of the results reveals that the amount of inhibitor used at 25 mg/l is effective for all three mediums. At this time, its corrosion protection efficiency is 94-98%.

As it is seen, reagent C-1 shows the best result (98%) in acidic medium. The reason for the significant decrease in the corrosion rate is due to the property of the reagent to hydrophobise the metal surface. More precisely, the reagent screens the active centres with high energy on the metal surface, isolating them from the aggressive medium and passivates the electrochemical corrosion process. Thus, the analysis of the results obtained

in all three mediums (neutral, acidic and alkaline), reveals that the reagent has a strong inhibitory property in terms of corrosion protection.

In order to determine the bactericidal properties of reagent C-1 in laboratory conditions, *Desulfomicrobium* and *Desulfovibrio desulfuricans* strains were used as a research object. Taking into account that sulfate-reducing bacteria can cause more corrosion aggressiveness in comparison with other bacteria, the destruction of sulfate-reducing bacteria by the reagent was studied. The selection of SRB is also related to the fact that during the termination of the life activity of these bacteria, it is possible to destruct the biocoenosis produced by other physiological groups of microorganisms.

The research of the effect of C-1 reagent on the incubation period of sulfate-reducing bacteria for fifteen days was carried out according to the following procedure. Taking into account intensive reproduction of SRB mainly in Postgate “B” nutrient medium, the experiments were carried out in this medium. Reagent-free and reagent-added mediums were used for comparison. First, 1 ml of the medium containing SRB was diluted with distilled water, then it was planted in Postgate “B” nutrient medium in pre-sterilized test bottles of 10 ml at a ratio of 1:9 and kept in a thermostat at 35°C for fifteen days. The bactericidal effect of the reagent taken for the experiment was observed daily and the results were given in the compiled table (table 4).

The presence or growth of sulfate-reducing bacteria in the studied medium can be determined visually based on the following indicators. The appearance of bacteria under a microscope was studied in an MBI-6 microscope.

1. The occurrence of coloured (black or white) deposits at the bottom of the test glass. 2. The occurrence of hydrogen sulphide. 3. The presence of living forms of sulfate-reducing bacteria. SRB grow intensively and synthesize biogenic hydrogen sulphide as a product of life activity and become black in colony form. However, when the chemical reagent added to the medium has a bactericidal effect, white deposit appears at the bottom of the test bottles, which in turn is a visual indicator of the suspension of the bacteria metabolism.

During daily observations, as a rule, black deposit is marked with sign “+”, and white deposit with conditional sign “-“ (table 4). The symbol “+” denotes the presence of bacteria, while the symbol “-“ indicates the absence of bacteria in the environment.

Table 4 The effect of reagent C-1 on the life activity of sulfate-reducing bacteria for 15 days

Concentration (g/l)	Incubation period during SRB, days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10	-	-	-	-	-	-	-	-	⊥	⊥	⊥	+	+	+	+
15	-	-	-	-	-	-	-	-	-	-	-	⊥	+	+	+
20	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SRB-free environment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SRB environment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The analysis of the mentioned signs is as follows. A positive sign means that sulfate-reducing bacteria were able to grow in the medium to which the reagent was added, or the reagent could not efficiently affect the life activity of the bacteria. In this case, as mentioned above, the bottom of the test bottle darkens. A minus sign, when the colour of the test bottles turns to a white background, means that the growth of SRB has stopped completely and the reagent has a high bactericidal effect. If blackening does not occur completely at the bottom of the test bottle and a part of it remains in the form of white deposit, it means that the reagent had a biostat effect, and this is indicated in the table with symbol ⊥.

As it is seen from table 4, at a concentration of 25 mg/l reagent C-1 has a high effect on the growth of SRB for fifteen days and completely stopped their growth from the first day, and it was confirmed by sign “-“ in this table. However, starting from day 9 at a concentration of 10 mg/l of C-1, day 12 at a concentration of 15 mg/l of C-1 and day 14 at a concentration of 20 mg/l of C-1, darkening of the bottom of the test bottles and growth of SRB were observed. Those days are indicated with “+” sign in the table.

At the end of fifteen-day experiment, the number of sulfate-reducing bacteria (cell number /ml) in reagent-free and reagent-based mediums was determined under a microscope (table 5).

Table 5 The effect of reagent C-1 on the number of sulfate-reducing bacteria during 15 days

C-1 concentration (mg.l ⁻¹)	0	10	15	20	25
Bacterial count (ml ⁻¹)	10 ⁷	10 ⁶	10 ⁴	10 ²	0

As it is shown in table 5, millions of SRB grow in reagent-free “Postgate-B” medium, which is taken for control (10⁷). The number of bacteria in the mediums with added reagent significantly decreased with increasing concentration of C-1.

The bactericidal effect of reagent C-1 was studied mainly by being observed for fifteen days and at the end it was studied by calculating the concentration of biogenic hydrogen sulphide generated in Postgate “B” medium. The occurrence of biogenic hydrogen sulphide in the medium was determined by the iodometric titration method. Both the concentration of biogenic hydrogen sulphide and the number of microorganism cells were determined daily. The amount of biogenic hydrogen sulphide formed in “Postgate-B” medium was calculated by titrating the reagent using the iodometric method.

The results of bactericidal effects of reagent C-1 based on the amount of biogenic hydrogen sulphide formed in the medium are given in table 6.

4. Conclusions

1. For the first time, the protection efficiency of a new reagent of organic origin in aggressive corrosion medium with neutral, acidic, alkaline and sulfate-reducing bacteria was investigated in laboratory conditions.

Table 6 Bactericidal effect of reagent C-1

	C-1 concentration (mg/l)	C _{H₂S} (mg/l)	Bactericidal effect Z (%)
With SRB	0	270	0
	10	110	59
	15	70	74
	20	50	81
	25	40	85
Non-SRB environment	0	34	-

Concentrations of 10, 15, 20 and 25 mg/l of the reagent were used in the research process.

2. The result of the experiments conducted in neutral, acidic and alkaline mediums revealed that the reagent has a high protective inhibitory property. In the concentration range of 10-25 mg/l of the reagent, the corrosion rate in the neutral medium varies between 0.0937-0.0390 g/m².h, the protection effect varies between 88-95%, the corrosion rate in the acidic medium varies between 0.1856-0.0265 g/m².h, the protection effect varies between 86-98%, and in an alkaline medium, the corrosion rate varies between 0.2048-0.0614g/m².h, and the protection effect varies between 80-94%.

3. The new reagent investigated in “Postgate-B” nutrient medium showed a high bactericidal effect against sulfate-reducing bacteria. Thus, at a concentration of 25 mg/l reagent C-1 highly affected on the growth of SRB for fifteen days and completely stopped their growth from the first day. However, the growth of SRB was observed starting from day 9 at 10 mg/l concentration of C-1, day 12 at a concentration of 15 mg/l and day 14 at a concentration of 20 mg/l.

4. At the end of the fifteen-day experiment, the number of sulfate-reducing bacteria (the number of cell/ml) was determined under a microscope in reagent free and reagent-based mediums. At this time, the number of bacteria varied as n=10⁶ at 10 mg/l, n=10⁴ at 15 mg/l, n=10² at 20 mg/l and n=0 at 25 mg/l. The bactericidal effect was between 59-85% at a concentration of 10-25mg/l.

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