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COMPARISON OF TWO WAYS OF THE METALS PROTECTION AGAINST CORROSION CAUSED BY OF *DESULFOVIBRIO DESULFURICANS*

Karamysheva N.N.[1], Semenov A.M.[2*], Vasilyev D.A.[1], Morozov A.V.[1], Ignatov A.L.[1]

[1]Federal State Budgetary Educational Institution of Higher Education Ulyanovsk State Agricultural Academy named after P.A. Stolypin. Ulyanovsk, 433431, Russia.
E-mail[1]: Natalikar@inbox.ru, dav_ul@mail.ru, alvi.mor@mail.ru, ignatovalecsei@yandex.ru.
[2]Department of Microbiology, Biological Faculty. Moscow State University named after M.V. Lomonosov. 119234. Lenin's Hills, 1, 12. Moscow, Russia. E-mail: *amsemenov@list.ru.

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ABSTRACT
The introduction simultaneously with the technological water during oil production the suspension of specific phage which attacks on *Desulfovibrio desulfuricans* and carry out the lyses the bacteria, allows protecting the metal from biocorrosion instead of the use of traditional chemical protectors.

Keywords: biological corrosion, sulfate-reducing bacteria, inhibitor, bacteriophage, bioprotection.

INTRODUCTION
In oil production industry the method of flooding of oil stratum by using of available natural waters is widely spread but that promotes to intensive growth of sulfate-reducing bacteria (SRB), *Desulfovibrio desulfuricans* in particular (Rozanova, Mehtieva 1969; Littman 1987; Drogaleva et al. 2013). It is known that in samples of such technological water taken for oil extraction up to 10^5-10^6 cell/ml of bacteria of D. *desulfuricans* can by found (Akshentseva 1991). Growth and accumulation of *Desulfovibrio desulfuricans* in such habitats causes biological corrosion of metal equipment that is a critical factor for durability of metal pipes. To suppress bacterial sulfate reduction (SR) usually used the chemicals are injecting into the oil stratum, for example, the solution of formalin that plays a role of a bactericide. Of course, the fact that the dilution of the formalin solution by stratal waters takes place, and the dose of formalin injected with water must reaches of 0.4 g/l. It is accompanied by economic expenses and technological the problems (Agaev et al., 1977). However, for a long time it was known that it is possible to control such problems by means of the bacteriophages which suppressing the growth of undesirable bacteria (Kamimura 1989). The authors isolated a phage and a specimen was produced readily consisting of bacteriophages with strict specificity to *Desulfovibrio desulfuricans* and a high degree of lytic activity (10^8). The idea to use the aqueous suspension of bacteriophages for a steel prevention and protection from biocorrosion had arisen. For this purpose, it was necessary to compare the effectiveness of the biocorrosion prevention by the traditional method -chemical substances and bio suppression (Iverson 1972; Mudretsova-Wyss 2001).

The aim of the study was to compare the use of formaldehyde as a bactericide and bacteriophages as a method of suppressing metal corrosion caused by SRB activity.

The following objectives were set to determine the main corrosion reason of the steel samples that are used to produce the equipment for the oil-extracting industry, in an experiment-ally created model imitating the corrosive media similar to oil production - to compare the efficiency of metal protection using the suspension of bacteriophage and formalin; - to determine the extent of corrosive damage of metal, comparing the rate of chemical corrosion and of bio-corrosion.

MATERIALS AND METHODS
In the experiments, the bacterial strain of *Desulfovibrio desulfuricans subsp. desulfuricans* from VKM B– 799 was used (www.vkm.ru, GCM -gcm.wfcc.info). The bacteriophages Ddu 48 and Ddr 57 at Ulyanovsk State Agricultural Academy named after P. A. Stolypin have isolated in the form of suspension and were used as suppressor agent. The samples of steel were taken in accordance with State Standards 9.905-82, 9.905-2007, which are using in oil industry for manufacturing oil pipelines, pump, and compressor pipes. Samples of oil were taken from the Aznakayevsky oilfield, Aznakayevsky of the region, Tatarstan Republic (Russia). The microcosms which are most the imitating conditions in the oil stratum

were created. The method of metallographic analysis to evaluate corrosive damage of the steel was used. This method allows determining the type of corrosion and distribution of corrosive damage in metals by means of comparison of newly obtained corrosive damage with the standard forms. The depth of the corrosion lesions on metallic section was specified by electronic microscopy, quantitative characteristics of corrosion were done gravimetrically.

Setup of the experiments: The samples of steel before testing were mechanically processed up to 7th class of cleanliness, the geometrical sizes and the weights were evaluated (Tab. 1). Surfaces of steel samples before corrosion tests are presented on figs. 1. Four types of the corrosion medium (CM) imitating the conditions which approximated the conditions in the oil stratum were prepared. CM # 1 - control, of distilled water 200 ml and 200 ml of crude oil were containing. CM # 2 contained 100 ml of 96-hour cultures of *D. desulfuricans* bacteria with a density of 10^6 cells/1 ml, 100 ml of water and 200 ml of oil. CM # 3 contained 100 ml of 96 h. cultures of *D. desulfuricans*, 100 ml of a specimen of he bacteriophages Ddu 48 with titer of 10^8 particles and 200 ml of oil. CM No. 4 consisted of 100 ml of 96 h. cultures of *D. desulfuricans* with the same cells density, 100 ml of distilled water, 4 ml of 10% formalin and 200 ml of oil. The number of *D. desulfuricans* if it required was determined by method of the most probable number of cells was using of McCreadi tables (GOST 39-151-83, [state system of certification of Russia]).

Table 1. Changes of metric indices of the steel samples after incubation in corrosive media*.

Corrosive medium	Area of steel samples, S, mm^2	Weight loss, (m_1-m_2), g	Corrosion rate, $V_к$ $g/m^2 \cdot h$	Efficacy of preparation, P (%)
CM №1 (oil – water, control)	766,9±34,5	0,0035±0,0012	0,0189±0,0060	–
CM №2 (oil – water – *D. desulfuricans*)	779,0±16,0	0,0111±0,0016	0,0594±0,0098	–
CM №3 (oil – water – *D. desulfuricans* – a specimen of the phage)	784,5±5,5	0,0040±0,0014	0,0214±0,0023	64,0
CM №4 (oil – water – *D.desulfuricans*-inhibitor (formalin)	766,8±36,6	0,0087±0,0013	0,0472±0,0076	20,5

* Deviation error: ± 0,03

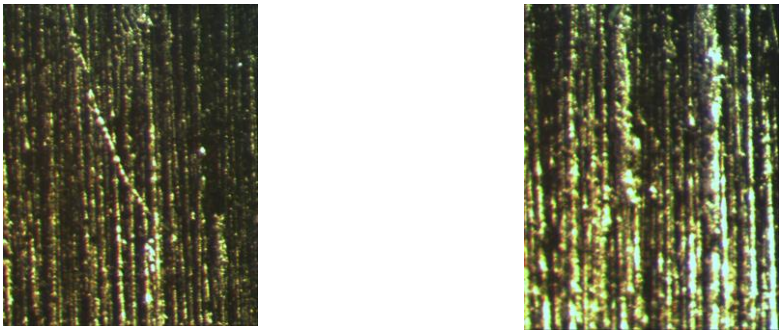

Fig. 1: The surface of the steel samples after mechanical processing (× 100).

Samples of the metal simultaneously have brought in all the vessels with the corrosion media at continuous a shaking and 25ºC. Duration of the experiment was 10 days. After the incubation the samples were taken from the emulsion, the remains of liquid were removed, and a visual inspection and microscopic examination of the surface were done. If necessary a surface of the samples was cleared from corrosion traces, using 10 % a solution of hydrochloric acid (d = 1,19g/sm^3) in accordance of GOST P 9.907-2007 Metals, alloys, coverings metal. Methods for removal of products of corrosion after corrosion testing. Microscopic examination of the steel surface was conducted using of MBF-10 microscope with the digital camera – DCM-310 eyepiece on metallic slice made for this purpose, and by looking at a profile of the corroded surface with magnification x-100 in the reflected light. The depth of corrosive damage on a metallic slice was determined by means of an eyepiece graticule and a micrometric screw of a microscope and was calculating like of the difference of metal thickness on a corroded site and the site which is slightly destroyed by corrosion. The rate of corrosion was calculated by a formula: Km = (m_1-m_2)/S*t, where Km - an indi-

cator of mass corrosion, dimension - g/(m^2/h); m^{1-} initial mass of a sample, g; m_2 - mass of a sample after tests; S - surface area of a sample, m^2; t - time of tests, hours.

Protective efficiency the specimen of bacteriophage from general corrosion (Pgc, %) was determined according to GOST 9.506-87 Inhibitors of corrosion of metals in water and oil media. Methods of protective ability determination, as the corrosion rate (g/m^2/h) of samples in the media without a bacteriophage (Vo) and in the medium with a bacteriophage (Vb) by a formula: Pgc = Vo – Vb/Vo • 100%. At calculation of the corrosion rate the relative error of measurements did not exceed 3 %.

Coefficient of suppression (K) as the indicator of the suppressive efficiency of phages was calculated by a formula: $K=lgn_0 - lgn_1/lgn_0$, where n_0–quantity of bacteria cells in control test, c/cm^3; n_1–quantity of bacteria cells in the test after bactericidal treatment, c/cm^3.

The experiments with bacteriophage Ddu 48 were carried out in three replications and repeated three times. However, in total the experiments were performed with two different bacteriophages Ddu 48 and Ddr 57 and of corrosive damage on metallic slices were defined not only on a steel surface, but also on cross-section a cut of samples.

RESULTS

After removal of steel samples from corrosive media at the visual examination of the oxidation of the metals were seen on the surface of control samples the rust in the form of reddish color deposits. Surfaces of all other samples were covered with a substance of black color, demonstrably the sulfide of iron. (Kushnarenko at al. 2012). At the microscopic examination of the control steel samples the uniform corrosion was detected on their surface (Fig. 2a), whereas the microscopic spots of corrosion in the form of irregular cavities of the various sizes were seen on the samples ejected from oil-water medium with the presence of bacteria *D. desulfuricans* (Fig. 2b). The corrosive spots were unevenly distributed on the metal surface, their depth has reached up to 1.6 microns. In the average by corrosion spots was affected up to 55.0% of the surface of steel. The surface of the steel samples subjected to testing in CM No.3 i.e., in the presence of bacteria *D. desulfuricans* and suspension of bacteriophages, least of all differed from the surface of initial samples, though there were the areas affected insignificantly by spots, with a total area about 11.0% (Fig. 2c). Samples after incubation in SM 4, i.e. with bacteria *D. desulfuricans* and a solution of formaldehyde, a corrosion spots also had, and the corrosion area reached to 45.0 % Fig. 2d).

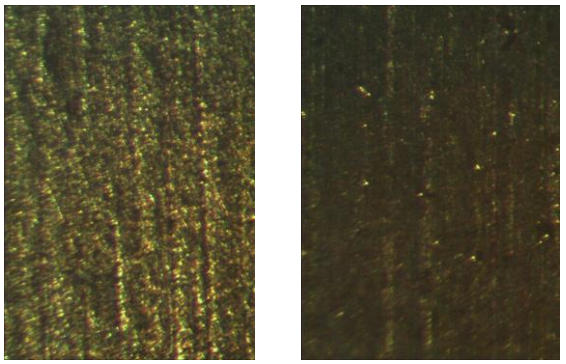

Fig. 2a: The surface of steel after incubation in the medium: oil – water (control, × 100).

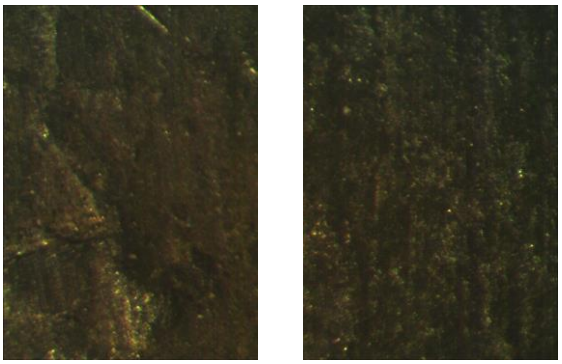

Fig. 2b: The surface of steel after incubation in the medium: oil – water - SRB (×100).

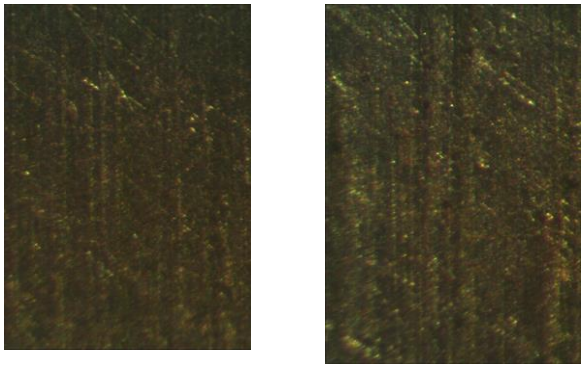

Fig. 2c: The surface of steel after incubation in the medium: oil – water - specimen of phages (×100).

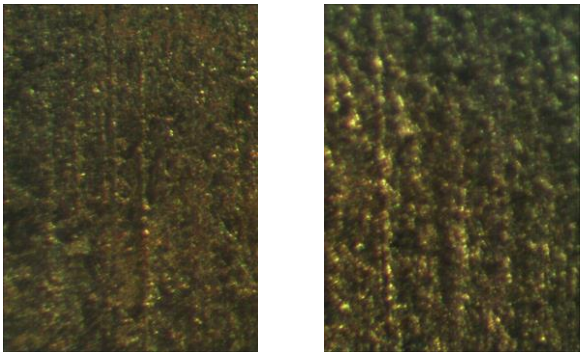

Fig. 2d: The surface of steel after incubation in the medium: oil – water - formalin (×100)

The data are provided in table 1 demonstrate that injecting the bacteriophages along with bacteria of *D. desulfuricans* into the corrosive media suppresses biocorrosion more effectively than the formalin.

The smallest loss of the weight and corrosion rate changing of steel samples are obtained in control (CM No. 1) and constitute 0.0035±0.0012 g and 0.0189±0.0060 g/m^2/h respectively. Corrosion in this case is caused obviously by the oxygen depolarization, where oxygen dissolved in the medium served as an oxidizer. The weight loss of the samples and corrosion rate change in CM 2 was by 2.1 times more then in CM 1 (0.0076g and 0.405 g/m^2/h, respectively), that is obviously by the existence of the sulfate-reducing bacteria. Therefore, by the difference of corrosion of the second medium and in the first one it is possible to evaluate biocorrosion because biological corrosion occurs more than twice intensive, than corrosion with the oxygen depolarization.

The changes of metric indices of the steel samples after incubation in corrosive media № 3 were quite close to values of samples from the first media. Excess of the steel values from SM № 3 over values of SM №1 were on 0.0005 г on weight and on 0.0025 g*m^2*h on intensity respectively. The bacteriophages added to the oil-water-SRB medium reduces the mean rate of biological corrosion from 0.0405g/m^2/h to 0.0025 g/m2/h, that is more than 16 times. The corrosion of samples after incubation in CM # 4 also was observed. The indices 0.0087±0.0013g and 0.0472 ±0.0076 g/m^2/h was lower in comparison with CM 2 but higher then after CM#3. The formalin solution added to the medium oil-water-SRB reduces the mean rate of biological corrosion from 0.0405 g/m^2/h to 0.0122 g/m^2/h, i.e. in 3.3 times. The efficiency of the biological method, using of bacteriophages for suppression on general corrosion, attain 64%, and a chemical bactericide – the formalin solution only 20.5%.

In order to confirm the efficiency of impact of the inhibiting action of the bicorrosion agents the number of *D. desulfuricans* the cells were counted in CM No. 2, 3 and 4 before and after ending of the experiment, tabl. 2. It was reveal that introduction the bacteriophages in CM #3 led to decreasing of the numbers of *D. desulfuricans* on three orders whereas injection of formalin had decreased of *D. desulfuricans* only one order.

Table 2. Number of viable bacteria *D. desulfuricans* in the corrosive media before and after termination of the experiment.

Corrosive medium	Beginning of the experiment	End of the experiment, 10 days
CM №1	-	-
CM №2	10^6 kl/ml	10^6 kl/ml
CM №3	10^6 kl/ml	10^3 kl/ml
CM №4	10^6 kl/ml	10^5 kl/ml

It is known that at the bacteriophage reproduction, a bacterial cell collapsed, and the quantity of phages goes up progressively with the death of cells. Detection of the viable cells of *D. desulfuricans* is explained first of all by probabilistic relationship of an encounter of a bacterium with a phage, i.e. the fewer bacteria cells remain, the lower the probability of an encounter of a cell and a phage particle. Effect of formalin leads also to elimination of a certain quantity of bacteria but considering a high adaptive ability of *D. desulfuricans* to the changing conditions over time formalin practically loses the suppressive properties for these bacteria.

According to the calculations, the suppression coefficient of corrosion by a bacteriophage is equal to 0.5 C/cm3, and the suppression of corrosion by of formalin is equal to 0.16 C/cm3. It means that the efficiency of a phage is 3.1 times higher than the efficiency of formalin.

DISCUSSION

The surface of steel used in oil production industry is exposed at least to two types of corrosion -the continuous and uniform, caused by oxygen depolarization and uneven corrosion, spots caused by the activity of *D. desulfuricans*. The using of bactericidal inhibitors are wide-spread method for the preventing of microbiological damage of materials and products as it is easier to perform and least expensive. In our studies, two ways of protection against corrosion were compared in created microcosms imitating the environment in a steel pipe with oil. There is a standard one the using of inhibitors - the formalin and second way is offered by us suspension of bacteria phages. When the formalin was added to the corrosive medium the rate of biological corrosion caused by the activity of *D. desulfuricans* decreases by 3.3 time whereas introduction of the bacteria phages reduced the corrosion rate by 16 time.

Taking into account that there is dilution by injected water in the oil stratum the formalin dose should be heightening constantly. Formalin belongs to cytotoxic poisons therefore its application impairs an ecological situation in the areas of oil production. The technique of obtaining bacterio-

phages of sulfate-reducing bacteria was tested by us multiply. Bacteriophages, being viruses, which intensively grow on bacteria and therefore circulate in all ecological niches, can provide an antibacterial effect for a long time, without degrading other members of the microbial community and non-injuring of others of the organisms. Suggested the type of biological, based on bacteriophage, the preparation significantly differs from traditional bactericidal preparations for bio-technologies. It is known from one cell after the lysis can be release from 10 to 100 new bacteriophages. Their advantage is a duration of action because of itself-reproduction bacteriophages.

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