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BIOCIDE TESTING FOR THE APPLICATION IN THE OIL AND GAS INDUSTRY***

1. INTRODUCTION

In the recent years, increased interest was observed in microbiological phenomena and their practical application in exploration and extraction of hydrocarbon deposits. Some biogenic processes are beneficial for the exploration works [23] and extraction (the activity of indicator microorganisms and their application in the assessment of prospectivity of the deposits, microbial enhanced oil recovery – MEOR, degradation of drilling wastes, etc.). However, excessive and uncontrollable development of microorganisms will result in microbiological contamination of a specific medium and changed composition. These changes occur due to processes of biodeterioration which translate into considerable economic loss. A necessary condition for the appearance and development of microorganisms is their presence in a specific environment of water and carbon source (tanks with drilling fluid, fuel tanks, oil pipelines, gas storage facilities, gas installations, etc.).

The phenomena of microbiological contamination related to oil and natural gas are quite an issue. Problems connected with chemical degradation and biodegradation may occur already at the stage of drilling the borehole. This is manifested, e.g. in deteriorated rheological parameters of water-based drilling fluids. Quite often, these unfavourable phenomena are accompanied by substantial increase in the hydrogen sulfide volume in the borehole, which is extremely dangerous for both the environment and the staff [16, 19]. Moreover, in some cases, intensive development of microorganisms may lead to rapid decomposition of drillling fluids, which in consequence contributes to significant economic loss and major complications in the drilling works.

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It should be noted that bacteria may penetrate to the drilling fluid by means of water (as the dispersive medium) or may originate from the confined groundwater layers [3, 8, 17, 18, 24].

Apart from the phenomena described, negative action of microorganisms is connected mostly with degradation of oil hydrocarbons [1, 9, 20, 26]. This process leads to higher oil density, sulfur content and changed viscosity. These adverse changes cause disturbances in the technology of oil extraction and processing, resulting in considerable economic loss. Also, there may be problems with storage of oil, products obtained from its processing [11, 14] and natural gas deposition in the underground geological structures [18]. Besides lower content of hydrocarbons in the oil, the adverse activity of microorganisms leads to corrosion of oil and gas transmission installations and production of undesirable substances (H_2S, polymers, organic acids, etc.) which not only have a negative impact on the parameters of oil and gas, but also may find their way to the environment [13, 14, 27]. The Table 1 demonstrates the extent of oil contamination in figures [5]. It should be noted that microorganisms get into the oil by means of formation water.

Table 1

Population of microorganisms and oil contamination

Population of microorganisms	Low contamination	High contamination	Pure product
Amount of bacteria [CFU/ml]	10^5	$10^6 – 10^8$	<50
Amount of fungi [CFU/ml]	$10^3 – 10^4$	$10^4 – 10^6$	<50

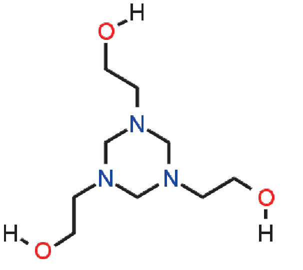
Another problem of biogenic nature is also the phenomenon of plugging (clogging), i.e. blocking of the rock pores by biomass which hinders the flow of the deposit media and lowers the permeability of the reservoir rock. It should be stated that permeability is one of the most important parameters which determine the appropriate process of hydrocarbon extraction [4, 10, 24]. The problems discussed above make it necessary to use effective substances in industrial applications. Also, essential is the assessment of their effectiveness with reference to isolated microorganisms [11, 19, 25].

The attempt to eliminate microorganisms is connected with application of chemical agents which demonstrate biogenic property, which, apart from the physical method is the most popular and effective technique of elimination of microbiological contamination. The selection of appropriate biocidal agents requires the consideration of factors which have an impact on the process of elimination of contamination in a specific environment. In the oil industry, mainly those agents are needed which shows the widest scope of action.

2. MATERIAL AND METHODS

The goal of this paper was to examine the preparations which have biocidal properties and to assess their usefullness in application in the oil industry. Frequently, triazine derivatives are used in the world industry. Performed laboratory examination covered tests for effectiveness of activity of a biocidal substance which is a component of typical biocides, and also assessment of usefullness of calcium and magnesium peroxides in elimination of microorganisms. In the tests hexahydro-1,3,5-tris(hydroxyethyl)-s-triazine was used. According to available literature [21] sym-triazine is a chemical product which has strong antibacterial properties. It is commonly used as preservative for lubricants and other industrial applications.

The examined antibacterial product, being derivative of triazine, is the result of reaction of formaldehyde with methylamine. The principle of biocidal action of sym-triazine consists in splitting off of a fragment of formaldehyde [21, 22]. Below, chemical formula of hexahydro-1,3,5-tris(hydroxyethyl)-s-triazine is demonstrated:



Formula of the compound: $C_9H_{21}N_3O_3$

Molar mass: 219.28 [g/mol]

Magnesium peroxide (MgO_2) occurs as white amorphous powder. In the presence of water MgO_2 decomposes and hydrogen peroxide is created which then undergoes exothermic disintegration to water and oxygen, in accordance with the following reactions:

$$MgO_2 + 2H_2O \rightarrow Mg(OH)_2 + H_2O_2$$

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Formula of the compound: MgO_2

Molar mass: 56.30 [g/mol]

Magnesium peroxide is used to reduce the amount of contamination in the groundwater and as a disinfectant in agriculture. According to the literature data [6, 7], magnesium peroxide is capable of inhibiting the SRB (*sulfate-reducing bacteria*) in anaerobic environment. It was proved that MgO_2 is able to inhibit the formation of biogenic hydrogen sulfide [22]. The application of commercially available ORC agent (oxygen release compound), consisting mostly of magnesium peroxide, inhibits the formation of biogenic H_2S. Chang demonstrated that MgO_2 in concentration of 0.4% has biocidal properties in relation to SRB, curbing the formation of H_2S for a period of approximately 40 days [6].

Calcium peroxide (CaO_2) occurs as amorphous powder, it disintegrates in analogical reaction as MgO_2 which further breaks down to active oxygen. Calcium peroxide found its application in monitoring the quality of water, including, e.g. control of production of hydrogen sulfide and counteraction of the development of plankton and bacterial biomass.

Calcium peroxide has several important properties which may be advantageous in controlling the growth of microorganisms. As a result of dissolution of calcium peroxide, the phosphate ion is removed due to precipitation of residue. Phosphate is one of the basic nutritients essential for appropriate development of microorganisms and the absence of this ion disturbs proper growth, and then, consequently, kills off the microorganisms. Some of the calcium peroxide in water environment is converted to reactive hydroxyl radical (HO*), which, by attacking the living cells of the microorganism by means of the radical mechanism, disturbs and damages the metabolic routes and causes death of the cells.

Formula of the compound:	CaO_2
Molar mass:	72.01 [g/mol]

As already discussed, in performed tests biocidal action of three substances was analyzed, including a typical biocide (hexahydro-1,3,5-tris(hydroxyethyl)-s-triazine) and two other substances showing biocidal properties (magnesium and calcium peroxide). Assessment of the activity of examined substances was made in respect of aerobic and anaerobic microorganisms and mildew fungi. In the tests, active suspensions of microorganisms were used and they contained aerobic and anaerobic strains of bacteria isolated from formation water, base water used in drilling technology, contaminated drilling fluid and extraction sludge (mainly from deposits of the peri-Sudeten Monocline and BMB deposits). Additionally, the suspensions were enriched with microorganisms from residues collected from the reservoirs in the refining installations. Each of the primary suspensions used in the tests, besides bacteria, contained also mildew fungi isolated from the bottom of liquid fuel tanks.

Appropriate agar media were prepared for the microbiological tests [2]. Quantitative determination of the aerobic bacteria was made on solid medium (pH 7.0) containing (gl^{-1}): meat extract – 3.0; peptone – 5.0; glucose – 1.0; agar – 15.0. Quantitative determination of the anaerobic bacteria was made on solid medium (agar columns) containing (gl^{-1}): yeast extract – 5.0; pancreatic hydrolyzed casein – 5.0; dextrose – 10.0 g; resazurin – 2.0; CH_3NaO_3S – 1.0; peptone – 10.0; $NaH(CH_2SCOO)$ – 2.0; NaCl – 5.0; agar – 20.0.

Quantitative determination of mildew fungi was made on solid agar medium (pH 6,6) containing (gl^{-1}): yeast extract – 5.0; glucose – 20.0; chloramphenicol – 0.1; agar – 15.0.

Tests were performed to select an optimal concentration of the tested substances, capable of producing the biocidal effect. The examined substance of set concentration (50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm and control sample) was added to the prepared active suspensions of microorganisms (volume of 50 ml). Then, after 10-day incubation in temperature of 30ºC quantitative tests were made, to determine the number of microorganisms in 1 ml of the liquid from the test sample (i.e. for each concentration of the biocidal substance).

3. RESULTS

Test results were listed in Tables 2–4. The goal of performed tests was to determine the biocidal activity of substances selected for application in the oil and gas mining industry. The effectiveness of action of the derivative of triazine and calcium and magnesium oxides was examined in order to eliminate the aerobic and anaerobic bacteria and mildew fungi which occur in formation water, drilling fluids, technological water, extraction mud and similar.

On the basis of performed tests (Tab. 2), it must be concluded that the solution of derivative of sym-triazine was the most effective in elimination of microorganisms, in comparison with other tested substances. Effective biocidal activity was observed in concentrations from 800 to 1000 ppm. The highest effectiveness was noted in elimination of mildew fungi. In those tests, at concentration of 1000 ppm, the number of fungi was reduced from the initial value of $1.1 \cdot 10^8$ CFU/ml to the low level of $2.0 \cdot 10^2$ CFU/ml. It was observed that the larger the dose of the biocidal substance used, the greater the antimycotic effect. Similar results are observed also in elimination of aerobic bacteria under the influence of the tested biocide. Whereas lower antibacterial activity can be seen in tests oriented at fighting the anaerobic bacteria. The addition of a dose of active agent ranging between 100 and 600 ppm produces identical effect. Only a dose of 800 ppm can considerably reduce the number of anaerobes.

Table 2

Tests for efficiency of hexahydro-1,3,5-tris(hydroxyethyl)-s-triazine antimicrobial activity (10-day incubation)

Concentration of derivative of sym-triazine [ppm]	Amount of aerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of anaerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of fungi in liquid medium [CFU/ml] ± SD
0 (control)	$\mathbf{6.8 \cdot 10^7 \pm 5.1 \cdot 10^4}$	$\mathbf{6.0 \cdot 10^5 \pm 3.9 \cdot 10^2}$	$\mathbf{1.1 \cdot 10^8 \pm 9.0 \cdot 10^4}$
50	$1.0 \cdot 10^7 \pm 2.3 \cdot 10^3$	$9.2 \cdot 10^6 \pm 1.1 \cdot 10^4$	$2.0 \cdot 10^7 \pm 3.7 \cdot 10^4$
100	$8.3 \cdot 10^5 \pm 4.4 \cdot 10^2$	$7.0 \cdot 10^4 \pm 2.0 \cdot 10^2$	$6.0 \cdot 10^5 \pm 4.6 \cdot 10^2$
200	$9.0 \cdot 10^4 \pm 1.0 \cdot 10^2$	$8.0 \cdot 10^4 \pm 0.00$	$1.2 \cdot 10^5 \pm 1.8 \cdot 10^2$
400	$1.1 \cdot 10^5 \pm 1.5 \cdot 10^3$	$8.0 \cdot 10^4 \pm 1.7 \cdot 10^2$	$1.0 \cdot 10^5 \pm 0.00$
600	$1.0 \cdot 10^5 \pm 9.1 \cdot 10^3$	$5.3 \cdot 10^4 \pm 9.3 \cdot 10^1$	$1.0 \cdot 10^4 \pm 0.00$
800	$4.4 \cdot 10^4 \pm 1.0 \cdot 10^1$	$2.2 \cdot 10^3 \pm 1.0 \cdot 10^1$	$2.5 \cdot 10^3 \pm 1.6 \cdot 10^1$
1000	$3.5 \cdot 10^2 \pm 7.0 \cdot 10^1$	$1.0 \cdot 10^2 \pm 0.00$	$2.0 \cdot 10^2 \pm 1.3 \cdot 10^1$

Another examined substance was calcium peroxide which was subject to analogical tests in concentrations from 50 to 2000 ppm (Tab. 3). This compound demonstrates a significantly weaker biocidal properties than a typical biocide. In concentration of 1000 ppm, substantial reduction of the number of examined microorganisms was noted, by an order of magnitude in comparison to the control sample. At increased concentration CaO_2 (2000 ppm), the biocidal effect was enhanced with reference to aerobic bacteria and mildew fungi and a fall was noted in the number of microorganisms by 2 orders of magnitude. A similar effect was achieved in the case of anaerobic bacteria, at increased concentration of CaO_2 to 2000 ppm.

Table 3

Tests for efficiency of Calcium peroxide antimicrobial activity (10-day incubation)

Concentration of CaO_2 [ppm]	Amount of aerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of anaerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of fungi in liquid medium [CFU/ml] ± SD
0 (control)	**$1.9 \cdot 10^6 \pm 2.1 \cdot 10^4$**	**$2.0 \cdot 10^6 \pm 0.0$**	**$6.0 \cdot 10^4 \pm 0.0$**
50	$1.8 \cdot 10^6 \pm 7.1 \cdot 10^4$	$2.0 \cdot 10^6 \pm 0.0$	$4.0 \cdot 10^4 \pm 1.0 \cdot 10^3$
100	$1.4 \cdot 10^6 \pm 2.8 \cdot 10^4$	$7.0 \cdot 10^4 \pm 2.0 \cdot 10^2$	$1.0 \cdot 10^4 \pm 0.0$
200	$1.6 \cdot 10^6 \pm 0.0$	$1.9 \cdot 10^6 \pm 0.0$	$1.0 \cdot 10^4 \pm 0.0$
400	$2.3 \cdot 10^5 \pm 1.4 \cdot 10^4$	$8.8 \cdot 10^5 \pm 4.2 \cdot 10^3$	$5.0 \cdot 10^3 \pm 1.6 \cdot 10^2$
800	$1.5 \cdot 10^5 \pm 0.0$	$4.1 \cdot 10^5 \pm 0.0$	$3.8 \cdot 10^3 \pm 1.5 \cdot 10^2$
1000	$1.2 \cdot 10^5 \pm 7.0 \cdot 10^4$	$4.1 \cdot 10^5 \pm 0.0$	$2.8 \cdot 10^3 \pm 2.7 \cdot 10^1$
2000	$1.0 \cdot 10^4 \pm 2.0 \cdot 10^2$	$2.0 \cdot 10^4 \pm 0.0$	$4.5 \cdot 10^2 \pm 1.0 \cdot 10^1$

Tests with the use of magnesium peroxide were also carried out in concentrations in the range of 50–2000 ppm (Tab. 4). Magnesium peroxide was used to stimulate the tested suspension at concentration of 1000 ppm, led to reduction of the number of aerobic microorganisms (bacteria and fungi) approx. a hundred times. The biocidal effect at this concentration was weaker, with reference to anaerobic bacteria. Approximately a five-time reduction in the number of the microorganisms can be noted when using the dose of 1000 ppm MgO_2. In higher concentration of MgO_2 (2000 ppm), the following decrease can be seen in the number of anaerobics in the test suspension.

Table 4

Tests for efficiency of magnesium peroxide antimicrobial activity (10-day incubation)

Concentration of MgO_2 [ppm]	Amount of aerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of anaerobic bacteria in liquid medium [CFU/ml] ± SD	Amount of fungi in liquid medium [CFU/ml] ± SD
0 (control)	$\mathbf{1.9 \cdot 10^6 \pm 2.5 \cdot 10^4}$	$\mathbf{2.0 \cdot 10^6 \pm 0.0}$	$\mathbf{4.7 \cdot 10^4 \pm 2.5 \cdot 10^3}$
50	$2.0 \cdot 10^6 \pm 3.2 \cdot 10^4$	$2.0 \cdot 10^6 \pm 0.0$	$3.7 \cdot 10^4 \pm 5.8 \cdot 10^3$
100	$1.4 \cdot 10^6 \pm 3.1 \cdot 10^4$	$1.6 \cdot 10^6 \pm 7.1 \cdot 10^4$	$1.0 \cdot 10^4 \pm 0.0$
200	$1.4 \cdot 10^6 \pm 8.0 \cdot 10^3$	$1.0 \cdot 10^6 \pm 0.00$	$1.0 \cdot 10^4 \pm 0.0$
400	$3.3 \cdot 10^5 \pm 1.3 \cdot 10^4$	$5.0 \cdot 10^5 \pm 2.1 \cdot 10^4$	$5.3 \cdot 10^3 \pm 1.2 \cdot 10^2$
800	$1.3 \cdot 10^5 \pm 1.5 \cdot 10^4$	$4.0 \cdot 10^5 \pm 3.0 \cdot 10^4$	$4.1 \cdot 10^3 \pm 2.3 \cdot 10^2$
1000	$4.7 \cdot 10^4 \pm 5.0 \cdot 10^2$	$3.7 \cdot 10^5 \pm 2.1 \cdot 10^4$	$3.6 \cdot 10^3 \pm 6.7 \cdot 10^2$
2000	$1.3 \cdot 10^3 \pm 1.0 \cdot 10^2$	$4.0 \cdot 10^4 \pm 2.0 \cdot 10^2$	$1.0 \cdot 10^2 \pm 0.00$

4. CONCLUSIONS

When analysing the results of tests for biocidal activity of the examined substances, the following conclusions can be made:

1. With reference to aerobic bacteria, the best biocidal effect was achieved after a solution of derivative of sym-triazine was added to the tested suspension in a range of concentrations from 800 to 1000 ppm. Another substance in turn which produces satisfactory effect by reducing the growth of bacteria was magnesium peroxide. Calcium peroxide turned out to be the least effective.
2. In tests conducted with reference to anaerobic bacteria, the highest effectiveness was shown for the derivative of sym-triazine. The examined substances (peroxide) demonstrated very similar effectiveness. However, it was much lower than the effectiveness of solutions based on sym-triazine.
3. The strongest effect on elimination of fungi was obtained in tests of solutions containing sym-triazine. The other substances (peroxides) were characterized by similar effectiveness in reducing the number of mildew fungi in the tested suspension.
4. The test results, particularly those performed for diversified concentrations of biocidal substance, hexahydro-1,3,5-tris(hydroxyethyl)-s-triazine, will be useful for elimination of contamination of microbiological origin which occurs in the oil and gas industry.

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