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Laboratory research on selection of effective antimicrobial substances and H_2S scavengers used in drilling fluid technology and underground gas storage

Badania laboratoryjne nad doborem efektywnych substancji biobójczych i neutralizujących siarkowodór dla potrzeb technologii płuczek wiertniczych oraz podziemnego magazynowania gazu

Anna Turkiewicz[1], Marek Janiga[1], Marcin Rogaliński[2]

[1]Oil and Gas Institute – National Research Institute, [2]Polish Oil and Gas Company

ABSTRACT: The article discusses the results of biocide tests for application in the oil and gas industry. This research was carried out with the use of active agents, such as: nano-silver particle suspension, and the solutions of two antimicrobial substances. The second part of the laboratory study was testing H_2S scavengers. Preparations recommended for drilling fluid technology and underground gas storage facilities were used. It should be noted that biogenic processes can largely cause the phenomenon of degradation of drilling fluids. As a result of these processes, drilling mud gets contaminated and loses its technological and rheological properties, making it incapable of fulfilling its role during drilling operations. All the tested scavengers were triazine products. In general, this agent in a solution acts in two ways. The application of triazine derivatives (three isomeric forms) is a good means of eliminating microorganisms from drilling fluid or formation water. These active agents have strong antimicrobial properties. On the other hand, these substances can also neutralise the hydrogen sulphide. The research enaNafta-Gabled determination of the effectiveness of the antimicrobial activity of the following substances: nano-silver particles, nano-Ag in combination with oxazolidine, and nano-Ag with a combination with glyoxal. The results of laboratory tests also allowed for a comparison of the efficiency of the action of individual H_2S scavengers. The first two tests were conducted in the range of nano-silver particles concentrations from 0.05 to 0.6% vol., while the next tests (i.e. with the application of nano-Ag/biocide) were carried out in the concentration range from 0.02 to 0.5% vol. Bacterial or fungal colony units (CFU) were used as a reference method for assessing the microbial water quality. The formation water came from a facility of underground gas storage (collective water – i.e. water from separators). In parallel tests, the number of bacteria was also determined in the contaminated water-based polymer drilling mud. The number of microorganisms in the tested samples was compared with the CFUs in control samples without biocide. The described research is part of a complex study intended to conduct biomonitoring of deposit environments and to eliminate bacterial contamination and sulphating of hydrocarbons, especially in stored natural gas. Industrial operations in this field make it possible to maintain the correct quality of stored gas and contribute to the improvement of exploitation. Selected effective substances will be used in the future in industry to reduce the content of biogenic hydrogen sulphide and to decrease a number of harmful microorganisms in drilling muds and formation waters.

Key words: silver nanoparticles, biocide, H_2S scavenger, microbial contamination, drilling mud, formation water, oxazolidine, glyoxal.

STRESZCZENIE: W artykule omówiono wyniki testów skuteczności działania biocydów dla potrzeb przemysłu naftowego i gazowniczego. Badania zostały przeprowadzone z zastosowaniem zawiesiny cząstek nanosrebra oraz roztworów dwóch produktów biobójczych. Druga część pracy dotyczyła testów skuteczności działania pochłaniaczy siarkowodoru wytworzonych na bazie triazyny. Użyto preparatów rekomendowanych do zastosowania zarówno w technologii płynów wiertniczych, jak i w obiektach podziemnego magazynowania gazu (PMG). Pochodne triazyny (trzy formy izomeryczne) stosowane w przemyśle mają silne własności bakteriobiobójcze w odniesieniu do skażonych płuczek wiertniczych i wód złożowych. Należy zaznaczyć, że procesy biogenne są w dużej mierze odpowiedzialne za biodegradację cieczy wiertniczych. W wyniku tego tracą one swoje własności technologiczne i reologiczne, a także nie spełniają określonych zadań w otworze wiertniczym. Testowane w ramach pracy neutralizatory są produktami chemicznymi, których działanie jest dwutorowe. Mają one jednocześnie zdolność eliminacji bakterii ze skażonego środowiska, jak również pochłaniają wytworzony w nim siarkowodór. Badania pozwoliły na określenie aktywności biobójczej następujących substancji: nanosrebra, następnie

Corresponding author: A. Turkiewicz, e-mail: *anna.turkiewicz@inig.pl*

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nanosrebra w połączeniu z oksazolidyną oraz nanosrebra w połączeniu z glioksalem. Testy laboratoryjne umożliwiły również porównanie efektywności działania poszczególnych neutralizatorów H_2S. W badaniach ukierunkowanych na obecność bakterii oraz grzybów określano liczbę JTK (jednostek tworzących kolonie) w próbkach testowych w odniesieniu do liczby mikroorganizmów występujących w próbce kontrolnej (próbka płynu bez substancji biobójczej). Badania te przeprowadzono równolegle w środowisku zainfekowanych płynów, tj. wody złożowej z obiektów PMG (wody zbiorczej – jest to woda pobierana z separatorów) oraz wodno-dyspersyjnej polimerowej płuczki wiertniczej. Omawiane prace badawcze stanowią część kompleksowych badań biomonitoringowych środowisk złożowych, które prowadzone są w celu zwalczenia skażenia mikrobiologicznego i jednocześnie eliminacji zasiarczenia węglowodorów, szczególnie w obiektach magazynowania gazu ziemnego. Badania te przyczyniają się do utrzymania prawidłowej jakości gazu magazynowanego w PMG i tym samym prowadzą do usprawnienia eksploatacji. Wytypowane skuteczne preparaty chemiczne zostaną w przyszłości wykorzystane w przemyśle do zmniejszenia zawartości biogennego siarkowodoru oraz redukcji liczby niekorzystnych mikroorganizmów w środowisku płuczek wiertniczych i wód złożowych.

Słowa kluczowe: nanocząstki srebra, biocyd, pochłaniacz H_2S, skażenie mikrobiologiczne, płuczka wiertnicza, woda złożowa, oksazolidyna, glioksal.

Introduction

The article discusses research results conducted with the use of biocides (including nano-silver particles) and H_2S scavengers recommended for drilling fluid technology and underground gas storage. Tests of the effectiveness of H_2S scavengers were performed, along with an analysis of the sulphur compound content in the gas samples coming from the degassing process of tested suspensions.

Research work related directly to the conditions of wells is part of a complex study intended to monitor the deposit environments and eliminate onerous phenomena which occur during exploitation.

Before starting the industrial operation (with the use of preparations such as biocides or H_2S scavengers), it is necessary to analyse the sulphur compounds in the stored natural gas and to investigate the selection of a specific hydrogen sulphide scavenger. Laboratory tests were performed on biocides and triazine-based H_2S scavengers recommended for use in underground gas storage facilities, and the most efficient substance was selected. Biocide testing is a very important element of research on contamination prevention (Williams et al., 2016; Płaza and Achal, 2020).

The new environmental protection requirements, as well as the need to reduce CO_2 emissions in the energy sector, resulted in increased natural gas consumption, meaning that it is crucial to ensure regular supplies thereof. Therefore, supply of natural gas, as the most eco-friendly fuel, and the appropriate storage facilities are a priority and key issue for the national economy. The assurance of energy reserves is also the subject of scientific studies (Blanko and Faaij, 2018). Some UGS, such as salt caverns, may be used not only for storing natural gas and liquid fuels, but there are also studies on selecting the appropriate underground facilities to be used in the future for hydrogen storage (Tarkowski and Czapowski, 2018).

One of the factors affecting safe operation of UGS facilities (apart from potential malfunctions of devices and systems) may be the biochemical process occurring underground or inside tanks or pipelines (Maurer, 1992; Staniszewska et al., 2017). Products of microbial metabolism and bacterial biomass may disturb proper operation of a UGS facility and may be the reason for biodegradation of drilling muds. Microorganisms, particularly prokaryotes, are characterised by flexible metabolism, and the number and types of the enzymes they produce are usually induced by environmental factors. Some specialised microorganisms survive and spread in extreme environments, such as underground gas storage and pipelines, where the availability of nutrients (macro- and microelements), as well as water, is limited (Ivanova et al., 2007; Bombach et al., 2015; Staniszewska et al., 2017).

Anaerobic bacteria dwell not only deep inside rock layers, but also in the depths of the oceans, forming organised assemblages which cooperate with one another. They actively participate in transformation of chemical complexes and element circulation in nature, such as carbon, nitrogen, sulphur and phosphorus. These elements circulate between ecosystems and fulfil a significant role in the environmental spread of these compounds and elements, favouring its functioning. The entire biosphere is filled with complex relations between individual microorganism genera and species. Lab-measured metabolic activity of individual bacteria, investigated separately, is different from that occurring in natural conditions, where one usually deals with a number of phenomena (such as symbiosis, competition, cooperation or synergy). The fact that the given environment is inhabited by numerous species, often interdependent, affects the course of numerous chemical and biochemical reactions (Brito et al., 2013; Braakman et al., 2017).

The issues related directly to the processes of biogenic hydrogen sulphide production in natural gas and petroleum deposit environments, as well as in underground gas storage facilities, are particularly important aspects of operation. Hydrogen sulphide, as one of the products of a microbiological reaction and transformation, as well as a product of chemical reactions in rocks, is a subject of interest for global petroleum companies,

as well as numerous scientific and R&D centres (Fugiel et al., 1979; Niewiadomska, 1994; Mc Govern-Traa et al., 1996; Tardy-Jacquenod et al., 1998; Myhr et al., 2002; Hemme and van Berk, 2017).

Bacteria adapted to environments with high concentrations of salt belong to various genera and species. They have been isolated from underground deposit brines (Chen et al., 2009), saline soils (Shi et al., 2012), as well as salt deposits (Chen et al., 2007). For instance, confined brines were found to contain the *Halobacillus salsuginis* bacteria, which are able to form endospores (dormant forms resistant to adverse environmental conditions). Saline soils with high alkalinity contain such bacteria as: *Bacillus, Halomonas* and *Litoribacter* (Shi et al., 2012). High diversity can also be found in salt deposits, where, among others, the following bacteria were found: *Acinetobacter, Arthrobacter, Bacillus, Halomonas, Micrococcus, Pseudomonas, Salinicoccus*, as well as *Streptomyces* (Chen et al., 2007). Bacteria utilising sulphur compounds and dwelling in high saline concentrations also include *Thiobacillus* (van der Wielen, 2006; Marlow et al., 2014), investigated in this study. Bacteria which reduce sulphates are able to produce hydrogen sulphide and prefer a high salt concentration in the environment are encountered in deposits and installations. They are mostly *Desulfovibrio, Desulfobacterium* (Tardy-Jacquenod et al., 1998; Cypionka, 2000; Kaksonen et al., 2006), as well as *Desulfococcus, Desulfosarcina and Desulfobulbus,* often isolated from salt layers, deposits and salt lakes (Foti et al., 2007). In favourable external circumstances, the aforesaid bacteria can produce biogenic H_2S in deposit conditions (as well as in other environments they are adapted to). Apart from sulphate-reducing bacteria (SRB), it is also worth mentioning bacteria which reduce sulfites. This group of microorganisms includes Clostridia. These bacteria also occur in salty environments, where they coexist with other bacteria, e.g. *Bacillus* (Humayoun et al., 2003). The processes discussed above are based on microbiological transformations of sulphur compounds, including reductions and the accompanying oxidation, where they are one of many types of chemical and biochemical transformations (Peck, 1966; Voordouw et al., 1992; Szewczyk, 2003) occurring in hydrocarbon deposit environments.

Individual microorganisms cooperate in their access to energy, and they exchange genetic information and jointly perform metabolic tasks, which allows access to new, hitherto-unavailable environments. An example of cooperation is the activity of SRB in the area where methane is produced in geological formation, with access to sulphates and in the presence of methanotropic bacteria. This process, based on anaerobic methane oxidation, is thus possible thanks to two groups of microorganisms, i.e. due to the presence and cooperation of methanotrophs and SRB. Methane is regarded as a very active molecule, but at the same time, it is a stable compound. Anaerobic methanotrophs are able to break down this molecule, releasing an abundance of electrons. In turn, SRB can utilise the released electrons and use their excess to reduce sulphates and transform them into sulphides (Brito et al., 2013; Braakman et al., 2017). These two groups of microorganisms are interdependent and coexist in deposit environments, as well as in oceanic depths.

Microorganisms were often isolated from drilling muds or formation waters of UGS (Mc Govern-Traa, 1996; Niewiadomska and Turkiewicz, 2003; Raczkowski et al., 2004; Iwanova et al., 2007; Turkiewicz, 2009; Bombach et al., 2015; Staniszewska et al., 2017). In the case of a salt cavern environment, all the signs of microbiological activity are extremely limited (Turkiewicz et al., 2013).

In general, the ability to produce various enzymes, typical of bacteria and archaea, allows them to colonise almost any abiotic surface, provided that at least trace amounts of water are present. Trace amounts of water accumulated in rock pores or condensation water precipitating from natural gas (in pipelines and installations) enable the growth of prokaryotic microorganisms.

Microorganisms and accumulated biomass not only cause damage to the technical materials by weakening their structure, but also produce volatile compounds, such as hydrogen sulphide (Myhr et al., 2002; Gutarowska, 2013; Bombach et al., 2015). Storage of natural gas in underground facilities, in natural conditions, creates numerous difficulties, resulting from changes in the gas composition (occurrence or increased amount of H_2S and mercaptans), with a simultaneous increase in the bacterial biomass weight. Gas sulphating in UGS facilities caused by biogenic processes necessitates preventive activities (Turkiewicz, 2009; Myhr et al., 2002; Niewiadomska and Turkiewicz, 2003; Raczkowski et al., 2004; Turkiewicz et al., 2011). The accumulation of excessive biomass, particularly in the case of rock pores, is dangerous, as it may initiate the process of the rock trap silting-up and therefore hinder the proper, cost-effective flow of hydrocarbons, in this case – the stored natural gas. In drilling muds, many species of bacteria exist that can initiate the biodegradation process of fluid ingredients, such as polymers. As a result of this process, the rheological parameters of drilling mud change drastically, and the degraded drilling fluid does not fulfil its role in the borehole.

Biocide testing methodology

The goal of this paper was to examine the substances which have biocidal properties and to assess their usefulness in oil industry applications. Biocides based on oxazolidine and glyoxal

were used against microbial contamination. A new substance included in the tests was antimicrobial silver nanoparticles. The performed laboratory examinations covered tests for the activity effectiveness of a biocidal substance being a component of typical biocides and assessment of the antimicrobial usefulness of nano-silver (applied in combination with biocides). These substances were tested for elimination of microorganisms occurring in drilling mud or formation water. In the tests, typical water-based potassium-polymer drilling mud and a mixture of formation waters from UGS were used. Oxazolidine and silver nanoparticles are chemical products that have strong antibacterial properties. These products are used as preservatives for some technical materials and in other industrial applications.

Assessment of the activity of the examined substances was made against aerobic and anaerobic bacteria, as well as fungal spores. In the tests, active microbial suspensions were used, and they contained aerobic and anaerobic strains isolated from formation water, base water used in drilling technology and contaminated drilling fluid (mainly from deposits of the Fore-Sudetic Monocline, Barnówko–Mostno–Buszewo deposit, and UGS). Each of the primary suspensions used in the tests, besides bacteria, also contained mildew fungi isolated from drilling fluids.

Appropriate agar media were prepared for microbiological tests (Atlas, 1997). Determination of the number of aerobic bacteria was made on a solid medium (pH 7.0) containing (g/l): meat extract – 3.0; peptone – 5.0; glucose – 1.0; agar – 15.0. Quantitative determination of anaerobic bacteria was made on a solid medium (agar columns) containing (gl^{-1}): yeast extract – 5.0; pancreatic hydrolysed casein – 5.0; dextrose – 10.0g; 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 fungal spores, and especially mould spores, was made on a solid agar medium (pH 6.6) containing (gl^{-1}): yeast extract – 5.0; glucose – 20.0; chloramphenicol – 0.1; agar – 15.

Tests were performed to select an optimal concentration of the tested substances capable of producing the biocidal effect. The examined substances of set concentration:

- CTX Nano AG MPG: 0.05–0.6% vol. (Tab. 1, 2),
- CTX Nano AG MPG + biocide based on oxazolidine: 0.02–0.5% vol. (Tab. 3, 4),
- CTX Nano AG MPG + biocide based on glyoxal: 0.02–0.5% vol. (Tab. 5, 6),

were added to the prepared acting suspensions of microorganisms (volume of 50 ml). After a 10-day or 30-day incubation at a temperature of 30ºC, quantitative tests were then made to determine the number of microorganisms in 1 ml of the liquid collected from the test samples (i.e. for each concentration of the biocidal substance and control sample).

H_2S scavenger testing methodology

Four H_2S scavengers (active substances – amine compounds, including triazine derivatives) were tested under laboratory conditions:

- T-04051,
- T-04059,
- T-04061,
- T-04071.

The effectiveness of the aforesaid preparations in the hydrogen sulphide absorption process was determined. The laboratory experiments included quantitative tests intended to determine the extent to which the tested preparations were capable of neutralising H_2S.

For analyses, the gas chromatography (GC) method was employed. Four test samples were prepared in 250 ml orange bottles with ground glass joints, each containing 200 ml of the tested suspension, i.e. deposit water with H_2S. The hydrogen sulphide neutralisation degree was assessed in comparison with a control sample (i.e. sample without the scavenger). Sample 1 contained the scavenger called T-04051 with a volume fraction of 0.5%, whereas the preparation interaction time was 16 hours. Sample 2 contained the scavenger T-04059 with a volume fraction of 0.5%, whereas the time of its interaction with the test liquid was also 16 hours. Sample 3 contained the scavenger T-04061 with a volume fraction of 0.5%, and the interaction time was the same – 16 hours. The final substance T-04071 was tested after the same interaction time with the same volume fraction and in the same manner as the previous substances.

After a specified incubation time for the test samples, each sample was degassed, i.e. the gaseous phase was separated from the liquid phase using a kit for degassing deposit waters and liquids. This method consists in measuring the volume of the gas separated from the deposit liquid and drawing it into a syringe. In order to do so, the test samples (in 250 ml glass bottles, connected tightly to the burette with a rubber hose) were heated in a water bath, whereupon the gas was removed at 60ºC. The degassing time was 40 minutes. The isolated gaseous phase, containing hydrogen sulphide, accumulated in the burette vessels.

A series of analyses were then made, measuring the content of hydrogen sulphide and other sulphur compounds using the gas chromatography method (GC). Before the analyses, the chromatographic system was validated. GC analyses of sulphur compounds were made using a dual-channel, valve gas chromatograph: AGILENT 7890 A (Fig. 1), Serial No. CN11331027, with ChemStation software, ver. B.04.03, and the following arrangement of columns and detectors:

- thermal conductivity detector (TCD), packed column Molecular Sieve 5A Ultimetal 9FT × 1/8IN × 2.00 (Supelco Analytical);

- flame photometric detector (FPD) capillary column DB-1, 60 m long with internal diameter of 0.32 mm (Agilent Technologies).

During the analyses, a nitrogen carrier gas was used with a constant flow rate of 3 ml/60 sec. A column temperature gradient of 30°C to 240°C was applied. A temperature of 30°C was maintained for five minutes, while the temperature accretion was 25°C per minute. The samples were dosed with valveless dispensers (although the system was also fitted with a typical split/splitless injector).

The working temperature of the TCD was 200°C, and that of the FPD was 250°C.

Fig. 1. Dual-channel, valve gas chromatograph AGILENT 7890A
Rys. 1. Dwukanałowy, zaworowy chromatograf gazowy AGILENT 7890A

Reference curves were determined for the following chemical compounds:

- hydrogen sulphide;
- COS (carbon oxysulphide);
- methyl mercaptan.

The stability of the chromatographic array was then checked using reference mixtures for individual sulphur compounds and, once the calibration curves were determined, individual assays were commenced (Chromatograf gazowy AGILENT 7890A, 2011; Kania and Janiga, 2011).

The measurement uncertainty for hydrogen sulphide was between 3 and 15% mol/mol, whereas for other sulphide compounds, it ranged from 2 to 5% mol/mol. After calibration, the chromatographic array was inspected daily, using a standard with the highest concentration of hydrogen sulphide (i.e. 50 ppm).

The control sample (without the scavenger) was the first to be degasified, and the resulting gas was injected into the chromatograph using a Hamilton syringe. A single amount of injected gas sample was 20 microliters. The result was calculated into the number of ppm units of the gas from degasification for each tested sample and was the arithmetic average of three chromatographic analyses.

After analysing the control sample, after a specified time of incubation, the GC method was used to analyse successive test samples containing a specific scavenger with a 0.5% volume fraction. For the hydrogen sulphide present in the control sample (and original samples, before adding the scavenger), a high signal result was recorded, i.e. 200 ppm H_2S.

Similarly, chromatographic standards were used to determine the content of the remaining sulphur compounds, such as COS (carbon oxysulphide) and methyl mercaptan (CH_3SH). As the chromatographic analysis was performed simultaneously for all the aforesaid sulphur compounds, it was possible to analyse not only the H_2S content in the tested sample, but also to monitor the content of other sulphur compounds, which can also become neutralised by a specific H_2S scavenger.

It should be noted that the processes investigated in this study are crucial from the perspective of exploiting natural gas deposits and the issue of underground gas storage. Hydrogen sulphide scavengers are currently used in the petroleum and gas industry together with biocides, which restricts the growth of unwanted microflora or completely eliminate the biogenic phenomena. Hydrogen sulphide contaminating the deposit environment reduces the quality of the stored material. H_2S penetrates natural gas via water (or brine) located in the deposit structure, where this chemical compound is generated by a biogenic process via microbiological reduction of sulphates. Therefore, it is particularly important to perform tests and to select the appropriate, effective substance.

Results of biocide testing

Test results are listed in Tables 1–6. The effectiveness of biocides and nano-silver action was examined in order to eliminate the aerobic and anaerobic bacteria and mildew fungi which occur in the formation of water and drilling fluid.

On the basis of the performed 10-day tests (Tab. 1), it must be concluded that the solution of silver nanoparticles was effective in elimination of microorganisms. Biocidal activity was observed at concentrations from 0.4 to 0.5% by volume. The highest effectiveness was noted in the elimination of mildew fungi. In these tests, at a concentration of 0.2%, the number of fungi was reduced from the initial value of 1×10^3 CFU/ml to a low level of 12 CFU/ml. It was observed that the larger the dose of silver nanoparticles, the greater the antimycotic effect. Similar results were also observed in the elimination of aerobic bacteria under the influence of the tested substance. Lower antibacterial activity can be seen in tests aimed at fighting anaerobic bacteria. The addition of a dose of active agent ranging

between 0.05 and 0.3% produced only a partial effect. In this test, only a dose of 0.4–0.5% could considerably reduce the number of anaerobes. The second test was performed after 30-day incubation. The results were similar (Tab. 2), as a concentration of 0.5% of silver nanoparticles led to the elimination of almost all microorganisms used in this test.

Another examined substance was oxazolidine-based biocide (tested together with nano-silver), which was subjected to analogical tests at concentrations from 0.02 to 0.5% (Tab. 3, 4). Both compounds demonstrated significantly better biocidal properties than the results of previous tests. At a concentration of 0.1–0.2% (after 10 days of incubation), either substantial reduction of the number of aerobic bacteria or its complete elimination was noted compared to the control sample (Table 3). Oxazolidine in combination with nano-silver particles was quite effective in the contaminated drilling mud and formation water environments.

Anaerobic bacteria in a solution containing oxazolidine and silver nanoparticles at a concentration of 0.1–0.2% were mostly eliminated. There were no viable bacterial cells in the solution (Tab. 4) at a concentration of biocidal substances of 0.3%.

Another test performed after a 10-day incubation concerned the efficiency of action of glyoxal and nano-silver against aerobic bacteria (Tab. 5). Generally, bacterial cells were killed in formation water at a concentration of biocidal substances of 0.2%. In the polymer drilling mud, complete elimination of living cells was achieved at a concentration of 0.3%.

The last test (Tab. 6) showed that at concentrations of 0.1–0.2% (glyoxal/nano-silver), the number of anaerobic bacteria decreased from a starting value of $1 \times 10^5 - 4 \times 10^5$ CFU/ml to a value of 1–15 CFU/ml. There was a trace number of anaerobic bacteria or no bacteria detected at concentrations of 0.2–0.3% in this experiment (test performed on contaminated formation water and polymer drilling mud).

Table 1. Test results for antimicrobial efficiency of CTX NANO-AG MPG in contaminated potassium-polymer drilling MUD exposure time: 10 days (the test against aerobes, anaerobes, fungal spores)

Tabela 1. Wyniki testu efektywności biobójczej CTX NANO-AG MPG w odniesieniu do skażonej potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 10 dni (test eliminacji tlenowców, beztlenowców, zarodników grzybów)

Sample designation	Concentration of nano-Ag	Aerobic bacteria	Anaerobic bacteria	Fungal spores
	[% vol.]	[CFU/ml]	[CFU/ml]	[CFU/ml]
D-1	0.05	1×10^3	9.5×10^3	1×10^3
D-2	0.10	2.4×10^2	5×10^3	1.5×10^2
D-3	0.20	40	3.3×10^3	12
D-4	0.30	–	4×10^2	–
D-5	0.40	–	10	–
D-6	0.50	–	–	–
D-7	0.60	–	–	–
K-1 (Control sample)	–	1×10^3	2×10^4	1×10^3

(–) – not found

Table 2. Test results for antimicrobial efficiency of CTX NANO-AG MPG in contaminated potassium-polymer drilling MUD exposure time: 30 days (the test against aerobes, anaerobes, fungal spores)

Tabela 2. Wyniki testu efektywności biobójczej CTX NANO-AG MPG w odniesieniu do skażonej potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 30 dni (test eliminacji tlenowców, beztlenowców, zarodników grzybów)

Sample designation	Concentration of nano-Ag	Aerobic bacteria	Anaerobic bacteria	Fungal spores
	[% vol.]	[CFU/ml]	[CFU/ml]	[CFU/ml]
D-8	0.05	1.3×10^4	1.1×10^5	9.7×10^3
D-9	0.10	1.9×10^3	9.0×10^4	1.6×10^2
D-10	0.20	1.5×10^2	7.1×10^4	90
D-11	0.30	1.2×10^2	3×10^3	34
D-12	0.40	–	1×10^2	–
D-13	0.50	–	2	–
D-14	0.60	–	–	–
K-2 (Control sample)	–	1×10^4	1×10^5	3×10^4

(–) – not found

Table 3. Test results for antimicrobial efficiency of CTX NANO-AG MPG and oxazolidine in contaminated formation water and potassium-polymer drilling MUD exposure time: 10 days (the test against aerobes)

Tabela 3. Wyniki testu efektywności biobójczej CTX NANO-AG MPG i oksazolidyny w odniesieniu do skażonej wody złożowej i potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 10 dni (test eliminacji tlenowców)

Sample designation	Total concentration of nano-Ag + oxazolidine (1:1)	Aerobic bacteria isolated from formation water	Aerobic bacteria isolated from drilling mud
	[% vol.]	[CFU/ml]	[CFU/ml]
W-15/D-15	0.02	7.0×10^4	1.4×10^5
W-16/D-16	0.04	6.0×10^3	6.7×10^4
W-17/D-17	0.06	1.7×10^2	1.0×10^3
W-18/D-18	0.10	–	10
W-19/D-19	0.20	–	–
W-20/D-20	0.30	–	–
W-21/D-21	0.40	–	–
W-22/D-22	0.50	–	–
K-3/K-4 (Control sample)	–	1.0×10^6	6.0×10^6

(–) – not found

Table 4. Test results for antimicrobial efficiency of CTX NANO-AG MPG and oxazolidine in contaminated formation water and potassium-polymer drilling MUD exposure time: 10 days (the test against anaerobes)

Tabela 4. Wyniki testu efektywności biobójczej CTX NANO AG MPG i oksazolidyny w odniesieniu do skażonej wody złożowej i potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 10 dni (test eliminacji beztlenowców)

Sample designation	Total concentration of nano-Ag + oxazolidine (1:1)	Anaerobic bacteria isolated from formation water	Anaerobic bacteria isolated from drilling mud
	[% vol.]	[CFU/ml]	[CFU/ml]
W-15/D-15	0.02	5.2×10^4	1.0×10^4
W-16/D-16	0.04	1.4×10^4	1.7×10^4
W-17/D-17	0.06	1.0×10^3	1.1×10^4
W-18/D-18	0.10	29	40
W-19/D-19	0.20	–	2
W-20/D-20	0.30	–	–
W-21/D-21	0.40	–	–
W-22/D-22	0.50	–	–
K-5/K-6 (Control sample)	–	1.0×10^5	4.0×10^5

(–) – not found

Results of H_2S scavenger testing

Below is a table presenting the results of a test using the gas chromatography method for the four hydrogen sulphide scavengers investigated in this study. The goal was to select the most effective scavenger for industrial application in environments contaminated with H_2S. It should be noted that to date, scavengers mainly based on triazine or quaternary amines have been used numerous times according to the technology developed at the Oil and Gas Institute – National Research Institute. The scavengers were used in industry with effective biocides in some domestic natural gas storage facilities. These operations led to limitation and stabilisation of low hydrogen sulphide content in the stored gas, as well as partial or total elimination of the harmful microorganisms in individual UGS wells or caverns.

The results of the laboratory tests (Tab. 7) reflect the effectiveness of the tested substances applied at a concentration of 0.5% by volume. Initial laboratory research results of implementation work carried out at another UGS facility enabled determination of the initial concentration of 0.5–2% by volume. Here, it should be noted that in further implementation activities on the domestic UGS, higher concentrations of the biocide/scavenger were used, i.e. > 5% vol. However, the experimental works on the facility started from low concentrations (i.e. 0.5–1% vol.) based on methanol.

A series of laboratory tests, including analyses of test samples, reflected the quantitative degree of hydrogen sulphide

Table 5. Test results for antimicrobial efficiency of CTX NANO-AG MPG and glyoxal in the contaminated formation water and potassium-polymer drilling MUD exposure time: 10 days (the test against aerobes)

Tabela 5. Wyniki testu efektywności biobójczej CTX NANO-AG MPG i glioksalu w odniesieniu do skażonej wody złożowej i potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 10 dni (test eliminacji tlenowców)

Sample designation	Total concentration of nano-Ag + glyoxal (1:1)	Aerobic bacteria isolated from formation water	Aerobic bacteria isolated from drilling mud
	[% vol.]	[CFU/ml]	[CFU/ml]
W-23/D-23	0.02	9.0×10^5	1.7×10^6
W-24/D-24	0.04	6.0×10^4	1.0×10^6
W-25/D-25	0.06	1.5×10^4	3.5×10^5
W-26/D-26	0.10	4.0×10^2	1.3×10^3
W-27/D-27	0.20	–	55
W-28/D-28	0.30	–	–
W-29/D-29	0.40	–	–
W-30/D-30	0.50	–	–
K-7/K-8 (Control sample)	–	1.0×10^6	6.0×10^6

(–) – not found

Table 6. Test results for antimicrobial efficiency of CTX NANO-AG MPG and glyoxal in contaminated formation water and potassium-polymer drilling MUD exposure time: 10 days (the test against anaerobes)

Tabela 6. Wyniki testu efektywności biobójczej CTX NANO-AG MPG i glioksalu w odniesieniu do skażonej potasowo-polimerowej płuczki wiertniczej czas ekspozycji: 10 dni (test eliminacji beztlenowców)

Sample designation	Total concentration of nano-Ag + glyoxal (1:1)	Anaerobic bacteria isolated from formation water	Anaerobic bacteria isolated from drilling mud
	[% vol.]	[CFU/ml]	[CFU/ml]
W-23/D-23	0.02	1.6×10^4	3.4×10^4
W-24/D-24	0.04	1.0×10^3	9.5×10^3
W-25/D-25	0.06	1.0×10^3	1.1×10^4
W-26/D-26	0.10	4	15
W-27/D-27	0.20	–	1
W-28/D-28	0.30	–	–
W-29/D-29	0.40	–	–
W-30/D-30	0.50	–	–
K-9/K-10 (Control sample)	–	1.0×10^5	4.0×10^5

(–) – not found

neutralisation compared to the control sample. The qualitative tests determined the growth or lack of growth of bacteria, which is directly related to the H_2S emission from the test samples.

Laboratory tests were applied to 4 test samples and control samples, with the following results:

1. TN-04051 – the test sample revealed the presence of H_2S in the gas from degassing, and the hydrogen sulphide content in the gas was 0.4744 ppm;
2. TN-04059 – no H_2S was found in the test sample;
3. TN-04061 – no H_2S was found in the test sample;
4. TN-04071 – the test sample revealed the presence of H_2S in the gas from degassing, and the hydrogen sulphide content in the gas was 0.0045 ppm;
5. Control samples (without scavenger) – H_2S content – 200 ppm.

According to the data above (200 ppm H_2S in the test sample), a total neutralisation with a fraction volume of 0.5% after 16 hours of exposure took place in the case of two preparations:

- TN-04059
  This scavenger caused neutralisation of the entire hydrogen sulphide. The test sample was found to contain carbon oxysulphide COS at – 0.6048 ppm, and no methyl mercaptan was found.
- TN-04061
  This scavenger also caused neutralisation of the entire hydrogen sulphide. As for other sulphur compounds, COS was detected – 0.0112 ppm. No methyl mercaptan was found. In test samples 1–4 and in the control sample, no other mercaptans and no dimethyl sulphide were found.

Table 7. Test results for effective operation of hydrogen sulphide scavengers – TN-04051, TN-04059, TN-04061, TN-04071 – exposure time: 16 hours; solutions of H_2S scavengers: 0.5% by volume

Tabela 7. Wyniki testu efektywności działania pochłaniaczy siarkowodoru – TN-04051, TN-04059, TN-04061, TN-04071 – czas ekspozycji: 16 godzin; roztwory pochłaniaczy H_2S: 0,5% obj.

Sample designation	Preparation name	Hydrogen sulphide content (H_2S)	Carbon oxysulphide content (COS)	Methyl mercaptan content
		[ppm]	[ppm]	[ppm]
P-1	TN-04051	0.4744	0.0488	–
P-2	TN-04059	0.0000	0.6048	–
P-3	TN-04061	0.0000	0.0112	–
P-4	TN-04071	0.0045	0.0352	–
P-5	Control sample (without scavenger)	200	0.9003	9.4617

(–) – not found

In general, two products: TN-04059 and TN-04061, demonstrated high and equal effectiveness in terms of hydrogen sulphide neutralisation.

Regarding the other investigated sulphur compounds (apart from H_2S), every test sample contained a small amount of COS. In this respect, TN-04061 displayed some advantage, as it provided the smallest amount of this compound, i.e. 0.0112 ppm, among all the samples analysed. For industrial use, two equal (in terms of their effectiveness) scavengers were particularly considered: TN-04059 and TN-04061. To sum up, it must be emphasised that all four tested scavengers proved to be highly effective in eliminating the hydrogen sulphide from the test samples.

Conclusions

1. The applied research methodology enabled assessment of the effectiveness of antimicrobial substances and H_2S scavengers in terms of hydrogen sulphide absorption and selection of the appropriate preparation for the potential industrial procedure.
2. Analysis results found that the preparation called CTX Nano-Ag MPG showed pronounced antibacterial and antifungal properties at concentrations of 0.4–0.5% vol. The experiments were conducted in contaminated environments, such as water-base polymer drilling mud and formation water (Tab. 1, 2).
3. Oxazolidine-based biocide in combination with nano-silver particles was effective against aerobic and anaerobic bacteria at concentrations of 0.1–0.2% vol. (Tab. 3, 4). Glyoxal-based biocide in combination with nano-Ag was effective against aerobes at concentrations of 0.2–0.3% vol. The same substances were effective against anaerobes at concentrations of 0.1–0.2% vol. (Tab. 5, 6).
4. Based on the results of laboratory tests (Tab. 7), it can be suggested that the best substances for potential industrial application were scavengers produced on the basis of the active substance heksahydro-1,3,5-tris(hydroxyethylo)-s-triazine), called TN-04059 and TN-04061.
5. Application of the scavenger and selected biocides can reduce the hydrogen sulphide content in the stored gas or drilling mud. Based on these satisfactory test results, industrial operations at UGS facilities or on other objects could be planned in order to continue the process of eliminating the hydrogen sulphide remaining in rock layers, stored gas and salt caverns.

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Anna TURKIEWICZ Ph.D.
Assistant Professor
at the Department of Microbiology
Oil and Gas Institute – National Research Institute
25 A Lubicz St.
31-503 Krakow
E-mail: *anna.turkiewicz@inig.pl*

Marek JANIGA M.Sc. Eng.
Assistant
at the Department of Geology and Geochemistry
Oil and Gas Institute – National Research Institute
25 A Lubicz St.
31-503 Krakow
E-mail: *marek.janiga@inig.pl*

Marcin ROGALIŃSKI M.Sc.
Cooperation Coordinator
Department of Geology and Exploitation
Polish Oil and Gas Company
25 Kasprzaka St.
01-224 Warsaw
E-mail: *marcin.rogalinski@pgnig.pl*