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Laboratory tests on the use of *Thiobacillus and Acidihiobacillus* species in the process of eliminating H_2S from formation waters and process waters

Testy laboratoryjne wykorzystania bakterii z rodzajów *Thiobacillus i Acidithiobacillus* do eliminacji H_2S z wód złożowych oraz wód technologicznych

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ABSTRACT: This article reports on a series of laboratory tests aimed at determining to what extent it is possible to neutralise hydrogen sulphide with H_2S-utilising bacteria from the genera *Thiobacillus* and *Acidithiobacillus* in the environment of formation water and process water. Four active bacterial suspensions were used for this study. The formation water was sourced from underground gas storage (UGS) areas and from currently exploited deposits. The water was taken mainly from natural gas reservoirs. Different sample incubation times were applied during the experiments, i.e. 1-day to 30-day incubation periods. The changes in H_2S content under the influence of bacterial cultures were measured in individual samples and compared to the hydrogen sulphide content in control samples. The percentage of changes in H_2S levels in the tested formation water samples were calculated. Further tests were conducted on contaminated process water. These served as 'base waters' used for preparing water-dispersible polymer drilling fluids. The changes in hydrogen sulphide content under bacterial action were also studied in these samples. The aim of this work was to compare the performance of *Thiobacillus* and *Acidithiobacillus* bacteria. The suspension of *A. thiooxidans* was by far the most effective in neutralising H_2S, followed by the suspension of *A. ferrooxidans*. The detailed results are included in the tabulated statements. Other suspensions of the genus *Thiobacillus* proved to be much less effective at neutralising hydrogen sulphide in the tested water samples. The article also discusses the results of tests carried out to show the combined effect of bacteria and a nitrate inhibitor (called BMF Bac 4), which is used in some deposits with nitrate-based treatment technology. This technology is used worldwide as an alternative to the use of biocides in reducing hydrogen sulphide content in reservoir media.

Key words: bacteria, hydrogen sulphide, sulphate, formation waters, process waters, biogenic processes.

STRESZCZENIE: Artykuł omawia badania laboratoryjne ukierunkowane na stwierdzenie, w jakim zakresie możliwa jest neutralizacja siarkowodoru przez bakterie z rodzajów *Thiobacillus* i *Acidithiobacillus* w środowisku wody złożowej i wody technologicznej. Woda złożowa pochodziła z obszarów PMG (podziemnego magazynowania gazu) oraz z obecnie eksploatowanych złóż; wody pobierano głównie ze złóż gazu ziemnego. W trakcie eksperymentów zastosowano zróżnicowany czas inkubacji próbek testowych: od inkubacji 1-dobowej do 30-dobowej. W poszczególnych próbkach określano zmiany zawartości H_2S pod wpływem oddziaływania kultur bakteryjnych w stosunku do zawartości siarkowodoru w próbkach kontrolnych. Obliczono procentową zmianę poziomu H_2S w badanych próbkach wód złożowych. Kolejne testy przeprowadzono na skażonych wodach technologicznych. Są to tzw. wody bazowe, służące do sporządzania wodno-dyspersyjnych polimerowych płuczek wiertniczych. W wodach tych również badano zmiany zawartości siarkowodoru pod wpływem działania bakterii. W ramach pracy dokonano porównania działania czterech zawiesin bakterii z rodzajów *Thiobacillus* i *Acidithiobacillus*. Zdecydowanie najbardziej efektywne okazały się bakterie *A. thiooxidans*, natomiast na drugim miejscu była zawiesina *A. ferrooxidans*. Szczegółowe wyniki zawarto w zestawieniach tabelarycznych. Pozostałe zawiesiny bakterii z rodzaju *Thiobacillus* użyte w pracy badawczej okazały się znacznie mniej skuteczne w neutralizacji siarkowodoru w badanych próbkach wód. Artykuł omawia również wyniki przeprowadzonych testów obrazujących łączny efekt oddziaływania bakterii oraz inhibitora azotanowego (o nazwie BMF Bac 4), stosowanego w niektórych złożach w ramach technologii *nitrate-based treatment*. Technologia ta znalazła zastosowanie na świecie jako alternatywa dla wykorzystania biocydów, w celu zmniejszenia zawartości siarkowodoru w mediach złożowych.

Słowa kluczowe: bakterie, siarkowodór, siarczan, wody złożowe, wody technologiczne, procesy biogenne.

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Introduction

The presence of sulphur in natural gas can interfere with the quality of gas, especially in underground gas storage (UGS). The objective of the study is to find out to what extent bacteria which oxidise sulphur compounds can reduce the level of hydrogen sulphide in the aquatic environment and can be used to reduce sulphation of extracted formation waters or process waters. Formation waters from deep layers are extracted, for example, together with natural gas in the operation phase of UGS facilities or during the operation of gas fields. Moreover, these waters accompany oil deposits and often contain significant amounts of hydrogen sulphide. H_2S can be generated upon microbiological processes or can result from chemical reactions. It can also be formed in a given deposit in a 'mixed' manner – i.e. in a biogenic way and as a result of chemical changes.

Process waters (base waters) serve as a dispersion medium for components of drilling fluids, among other uses. Their composition is therefore very important, as the purity of this medium determines the microbiological state of the water-based liquids. If sulphate-reducing bacteria (SRB) are present in the water, the drilling fluid may become contaminated, causing it to degrade rapidly, the pH to change, and the biogenic hydrogen sulphide content to increase. When the base water contains alarge number of other bacteria or fungal spores (mainly mould), biomass increases in the drilling fluid, which changes many of the fluid's parameters. In metabolic processes, microorganisms consume fluid components, mainly polymer compounds (thus changing their density, viscosity, structural strength, and reactivity), which largely hampers the proper course of the drilling process, sometimes even making further work impossible. Rapid degradation of the drilling fluid means it must be replaced, which in turn increases the amount of industrial waste.

One of the products of biogenic reactions is hydrogen sulphide – a very negative factor from the operational point of view. This compound is destructive to the drilling fluid and the equipment. Strong reducing reactions which involve the secretion of H_2S can also take place in formation or waste waters. The resulting H_2S is accumulated in formation waters in a free or bound form. Full saturation of formation waters with hydrogen sulphide in extreme cases may even reach 4000 mg/l. Its content in water and gas is highly variable.

It should be noted that an excessive amount of the H_2S in a deposit is oxidised. The substances with which water comes into contact when flowing through geological layers, including gasses generated through various chemical and biological processes, are dissolved in formation water.

Waste brine deposits in the form of oil mining wastewater contain a number of different types of sediment particles and crude oil, other than salt. They are known to show a negative impact on the environment, thus inhibiting biological self-cleaning processes of water reservoirs. Such wastewater often requires the coagulation of pollutants, and some effluents of this type are re-injected into geological formations – after prior analysis – and subjected to chemical stabilisation (to avoid harmful changes in the deposit space). Such water is purified of oil, slurries, and other ingredients. This makes it possible to safely remove pollutants from the oil industry without harming the environment. This problem is very important due to the huge amounts of this type of wastewater generated during operation. Also, the injection of brines must be properly controlled so as not to have a negative impact on the chemical composition and purity of water lying in deeper horizons. In addition to oil residues, emulsions (or crude oil adsorbed on the sediment surface or onto solid particles suspended in the liquid), the waste sediment contains fragmented rock particles that can be separated from water using sedimentation.

In the practice of oil mining, waste formation water can be initially mechanically purified using various types of traps that catch sludge and sand; emulsion separation processes are also carried out. Dispersants can multiply the rate of bacterial decomposition of admixtures of oil and hydrocarbons in formation water.

Sulphur compounds, which occur in water extracted through the process of natural gas extraction, get into the aquatic environment in the form of sulphates, sulphides, and hydrogen sulphide. The maximum concentration of hydrogen sulphide depends on the geological structure of the deposit and its hydrogeological properties. The decisive role in these conditions is played by biological sulphate reduction processes using SRB (anaerobes); as mentioned above, the source of organic matter for bacteria in metabolic reactions is a bituminous substance. The environmentally toxic components of formation waters include hydrogen sulphide and phenols, which are highly poisonous to aquatic organisms. These substances hinder water regeneration, which occurs thanks to certain aquatic microorganisms. In the past, purifying water from hydrogen sulphide entailed a number of technological difficulties, resulting from the chemical nature of hydrogen sulphide. Currently, there are many methods for removing hydrogen sulphide and acid gasses from natural and shale gas (Schieman, 1999; Salma et al., 2001; Abishek, 2006; Mokhatab et al., 2015; Kapusta et al., 2021; Weiland and Hatcher, 2021).

Microorganisms play an important role in the transformation of sulphur, mainly in its oxidation and reduction. Under certain conditions, one group of microorganisms may be more active than another, whereby sulphates, sulphites, or other sulphur compounds may accumulate or be destroyed. Total or partial reduction of sulphur compounds, accompanied by the formation of H_2S, is very important in nature. It occurs under anaerobic conditions, mainly in the water of rivers and lakes, swamps, marine sediments, sulphur and gypsum deposits, hydrocarbon and bitumen deposits, and hot springs. Considerable amounts of hydrogen sulphide in nature result from the activity of this anaerobic group of bacteria. The sulphate reduction can be presented in total according to the following formula:

$$4\ H_2 + SO_4^{2-} \rightarrow H_2S + 2\ H_2O + 2\ OH^-$$

One of the main SRB – *Desulfovibrio desulfuricans* – is capable of using organic compounds of various chemical structures, from hydrocarbons and fatty acids to alcohols and other substrates (Cypionka, 2000).

These bacteria have the ability to reduce not only sulphates, but also sulphites, various polysulphides, and colloidal sulphur (except for crystalline sulphur). In the sulphate reduction process, sulphur is reduced to sulphides and hydrogen is oxidised to water. Carbon from organic substances is oxidised to carbon dioxide. Energy from this process is used for growth, reproduction, and biosynthesis reactions in bacterial cells. SRB are a source of microbial hydrogen sulphide. This group of microorganisms is often found in many deposits around the world (Prajapat et al., 2019). One of the methods for fighting biogenic contamination is to apply a biocide or H_2S scavenger. In the global oil industry, nitrate-based treatment is also used (Xue and Voordouw, 2015) in order to inhibit reduction reactions in favour of the oxidation processes of reduced sulphur compounds and to activate a group of microorganisms beneficial for a deposit.

Microbial activity is present everywhere, including oil and gas reservoirs. The archaea and bacteria isolated from these reservoirs are physiologically very diverse. Microorganisms living in formation waters or base waters can be considered both in terms of their harmful role in the operation process and negative impact on devices and reservoirs, as well as their undoubtedly positive significance for the course of specific biochemical processes (Kamalakshi and Bhagobaty, 2021). For these reasons, it is very important to properly approach the problem of microbiological contamination to enable natural reactions of removing the accumulated hydrogen sulphide from the rock layers and their water content. Bacteria that live in water can use nutrients available at very low concentrations. These bacteria can float freely in the aquatic environment or settle on solid substrates. Thionic bacteria, which constitute the subject of this study, occur individually or in colonies. The best-known group are from the genus *Thiobacillus* – whose species show great diversity in terms of physiological properties (Wargin and Marchelek, 2015) – and bacteria of the genus *Acidithiobacillus*. The species *Acidithiobacillus thioxidans*, *Thiobacillus thioparus*, and *T. denitrificans* are particularly active in the transformation of sulphur compounds. These bacteria obtain energy needed for the assimilation of CO_2 and their own organic substances from the oxidation of reduced sulphur compounds to sulphates or sulphuric acid (Das et al., 1993; Holt, 1994; Kotowski and Burkowska, 2009; Tang et al, 2009; Tian et al., 2017).

Materials and methods

Microorganisms used in the research

The following sulphur-utilising bacterial suspensions were used in the research: *Thiobacillus thioparus*, *Thiobacillus denitrificans*, *Acidithiobacillus thioxidans*, and *Acidithiobacillus ferrooxidans*. The rationale behind choosing these particular species in this study is provided below. Sulphur bacteria are found not only in sulphur and sulphide deposits and the waters accompanying hydrocarbon deposits, but in copper deposits as well. The metabolic activity of sulphur bacteria leads to local acidification of the environment and dissolution of sulphide minerals. This results in the mobilisation of minerals of sulphur, iron, and copper in the groundwater. The process of forming secondary minerals (such as gypsum or malachite) and the accumulation of elemental sulphur also take place under these conditions (Matlakowska and Piotrowska, 2018). The reduced sulphur compounds are used by bacteria as sources of energy and/or electrons in the dissimilatory oxidation processes, and sulphates are the final products of this process.

In a study by Badr et al. (2014), *Thiobacillus thioparus* (DSMZ 5368) was used as a sulphur oxidising bacterium for producing elemental sulphur and sulphates as the products in the oxidation of a sulphur compound: methanethiol. In another article (Ramirez et al., 2009), *T. thioparus* was proposed for removing H_2S contained in air.

Under anaerobic conditions bacteria of the genus *Thiobacillus denitrificans* can use nitrates present in water, reducing them to molecular nitrogen whilst oxidising reduced sulphur compounds to sulphates (Hiszpańska et al., 2001; Kotowski and Burkowska, 2009). *T. denitrificans* uses nitrates as an electron acceptor, transforms them into nitrogen, and uses their oxygen to oxidise the sulphur.

Bacteria belonging to the genus *Acidithiobacillus thioxidans* represent the gamma subclass of Proteobacteria. They use sulphur as their primary energy source. Biofilms of *A. thiooxidans* have proved to exhibit high removal capacity in the process of H_2S elimination (Aroca et al., 2007).

In another study (Das et al., 1993), the bacterium *Acidithiobacillus ferrooxidans*, which oxidises reduced sulphur compounds, was used to desulphurise petroleum oil and gas. The reaction was carried out in a closed vessel containing a substrate mixed with a bacterial suspension. The significance of the H_2S oxidising activity of *A. ferrooxidans* was discussed.

The Proteobacterium *A. ferrooxidans* is a model for extremely acidophilic microorganisms that are capable of aerobic and anaerobic growth on elemental sulphur coupled to oxygen and a ferric iron reduction, respectively (Osorio et al., 2019). For the reasons given above, these four species of bacteria were used in laboratory tests in this work.

The individual bacterial suspensions contained approximately 10^4 bacterial cells per millilitre of culture medium. The pH value of the test fluids varied. The collective formation water with the addition of a specific bacterial suspension (volume ratio: 3:1) was characterised by the following pH values:

- test fluid with *T. thioparus* suspension – initial pH = 7.5
- test fluid with *T. denitrificans* suspension – initial pH = 7.0
- test fluid with *A. thiooxidans* suspension – initial pH = 3.9
- test fluid with *A. ferrooxidans* suspension – initial pH = 3.5

The same initial pH values also applied to the test fluids based on the process water (also called base water). This water, coming from an intake located in the area of the drilling rig, mainly from the Barnówko–Mostno–Buszewo oil field, was tested in combination with individual bacterial suspensions (by volume 3:1).

Research methodology

The samples of formation water with bacterial suspension were placed in sterile 250-ml bottles, where 150 ml of the volume was taken up by water, and 50 ml by the suspension of specific bacteria. The first test (Table 1) investigated changes in the hydrogen sulphide content in the bacterial test samples (WZ-1–WZ-4) in relation to the level of hydrogen sulphide in the control sample (K-1). Hydrogen sulphide in the formation water environment was determined iodometrically after 5 and 10 days of incubation at 30°C.

The method consists in determining the content of S^{2-} sulphide ions in solution by titrating samples with sodium thiosulphate in the presence of iodine, and then in the presence of starch as an indicator (Fugiel et al., 1979; Raczkowski, 1981; Pawlak and Pawlak, 1999; Moore and Spitler, 2003). It is an oxidation-reduction method in which iodine oxidises hydrogen sulphide to sulphur. Prior to the measurement, sulphide ions are absorbed from the test solution using an acidified cadmium acetate solution. The content of H_2S and sulphides dissolved in water is determined from the loss of iodine in a specific amount of the standard iodine solution, according to the following reaction:

$$H_2S + I_2 \rightarrow 2H^+ + 2I^- + S$$

Sulphide ions were absorbed directly during sampling, and then, after delivery to the laboratory, the samples were titrated with 0.05% sodium thiosulphate ($Na_2S_2O_3$) solution.

The second test (Table 2) involved the change in H_2S content after a longer incubation time. Analyses were performed after 20 and 30 days of incubation, using the same bacterial suspensions as in the previously described test. The results were compared with the level of hydrogen sulphide in the control sample K-1, which is presented in the table.

Another laboratory test was performed on process water contaminated with hydrogen sulphide, which was the basis for the drilling fluid production (samples WT-1–WT-4). Table 3 shows the results of iodometric analysis after 5 and 10 days incubation of the test samples. Table 4, on the other hand, presents the hydrogen sulphide content after 20 and 30 days of incubation of SRB in the process water environment. The results of the analysis were related to the level of H_2S in the control sample K-2 (with no bacteria).

As a part of the research work, an analysis of the total effect of bacterial suspensions with the nitrate inhibitor BMF Bac 4 were also carried out. It should be mentioned here that nitrate agents (sodium and/or potassium nitrate) are in many cases used in industrial technologies called nitrate-based treatment. Not only are they aimed at reducing the content of hydrogen sulphide in the formation media, but they also have an economic aspect (by implementing the Enhanced Oil Recovery method with the use of nitrate agents). The tests were conducted with the use of four bacterial suspensions together with the inhibitor at a concentration of 0.2% by volume (Tables 5–6). This concentration was selected in previous studies for the needs of the domestic oil industry. Contaminated formation water treated with the inhibitor at concentrations ranging from 0.05–0.5% by volume was tested. In the course of the laboratory work, a concentration of 0.2% by volume was selected as sufficient in the process of H_2S neutralisation. The tables present the combined effect of the nitrate agent and bacterial suspensions on the content of hydrogen sulphide in the aggregate formation water samples. Samples WZ-I-1–WZ-I-4) were analysed by the iodometric method after 24 and 48 hours of incubation. In the course of further research, the effects of the nitrate inhibitor and bacterial suspensions after longer incubation times were analysed. The samples labelled WZ-I-5–WZ-I-8 were subjected to 10 and 30 days of incubation. Then the level of hydrogen sulphide was measured for the above test samples. The results of the analyses (Tables 5–6) were compared with corresponding results for the control samples, i.e. the formation water samples without added bacterial suspensions.

Test results (Tests 1–4)

The results of laboratory tests to determine the effectiveness of sulphur-oxidising bacterial suspensions in the process of H_2S elimination are summarised in Tables 1–4. The study was divided into two stages: tests performed with microbiologically contaminated aggregate formation water and with contaminated process water. Aggregate formation water includes material from several production wells from eight gas deposits at Monoklina Przedsudecka (geological unit in south-western Poland) and from UGS facilities. The hydrogen sulphide content was measured after adding SRB to the formation water, then the samples were incubated and compared with the control fluid (without any added bacterial suspension). As shown by the results, after 5 days and 10 days of incubation, the suspension of *Acidithiobaciluus thiooxidans* was the most effective of the tested samples (Table 1, test samples labelled WZ-3). After the set time of interaction in formation water, approx. 27.24–27.74% of the hydrogen sulphide was neutralised in comparison to the control samples. The second most effective suspension was *A. ferrooxidans* suspension, in which the hydrogen sulphide content decreased by 7.23–7.57% (samples marked WZ-4). The other two suspensions were not effective after 5 and 10 days of incubation.

The second test involved 20- and 30-day incubation periods of the test samples (Table 2). The suspension of *A. thiooxidans* bacteria deserves particular attention. As a result of its action, H_2S was neutralised at the level of 32.05–32.25%. The *A. denitrificans* suspension reduced the content of hydrogen sulphide in the tested fluid by about 8.89–12.69%. The remaining two suspensions did not show any effect in the environment of formation water. There was a very slight decrease in H_2S, from the initial value of 85.5–85.9 mg S^{2-}/dm^3 to the value of 83.1–83.4 mg/S^{2-}/dm^3 after 30 days of incubation. This corresponds to an insignificant reduction of 2.91%–3.26%.

The third and fourth tests were related to the analysis of process water. This is water from the well in the area of the drilling rig. As mentioned, this water is used in industrial conditions as a base for producing the drilling fluid. Some

Table 1. Changes in biogenic H_2S content in formation water samples after adding bacterial suspensions (bacteria representing the genera *Thiobacillus* and *Acidithiobacillus*)

Tabela 1. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody złożowej pod wpływem zastosowania zawiesin bakteryjnych (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension	H_2S content in test samples [mg/dm³] (collective formation water)	
		5-day incubation	10-day incubation
WZ-1	*Thiobacillus thioparus*	85.1	85.0
WZ-2	*Thiobacillus denitrificans*	83.4	83.4
WZ-3	Acidithiobacillus thiooxidans	62.0	62.5
WZ-4	*Acidithiobacillus ferroxidans*	79.6	79.4
K-1 control sample (without any bacterial suspension)	–	85.8	85.9

Table 2. Changes in biogenic H_2S content in formation water samples after adding bacterial suspensions (bacteria representing the genera *Thiobacillus* and *Acidithiobacillus*)

Tabela 2. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody złożowej pod wpływem zastosowania zawiesin bakteryjnych (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension	H_2S content in test samples [mg/dm³] (collective formation water)	
		20-day incubation	30-day incubation
WZ-1	Thiobacillus thioparus	83.9	83.1
WZ-2	Thiobacillus denitrificans	83.5	83.4
WZ-3	Acidithiobacillus thiooxidans	58.1	58.2
WZ-4	*Acidithiobacillus ferroxidans*	77.9	75.0
K-1 control sample (without any bacterial suspension)	–	85.5	85.9

Table 3. Changes in biogenic H_2S content in base water samples after adding bacterial suspensions (bacteria representing the genera *Thiobacillus* and *Acidithiobacillus*)

Tabela 3. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody bazowej pod wpływem zastosowania zawiesin bakteryjnych (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension	H_2S content in test samples [mg/dm³] (base water used in drilling technology)	
		5-day incubation	10-day incubation
WT-1	Thiobacillus thioparus	39.7	37.0
WT-2	Thiobacillus denitrificans	38.4	38.1
WT-3	Acidithiobacillus thiooxidans	21.6	20.9
WT-4	*Acidithiobacillus ferroxidans*	26.8	27.1
K-2 control sample (without any bacterial suspension)	–	40.1	40.5

waters contain SRB; only part of the base water extracted from oil or gas areas contains hydrogen sulphide. Therefore, for laboratory tests, water was additionally contaminated with SRB to consider more extreme conditions that may occur during drilling. The results of these studies are presented in Tables 3 and 4. In this case, the *A. thioxidans* bacterial suspension proved to be highly effective, even after a 5-day incubation period. After this time, a 46.13% neutralisation of hydrogen sulphide took place. On the other hand, after 10 days of exposure to the active suspension of *A. thioxidans* bacteria, an even more significant decrease in the level of H_2S in water was obtained: by about 48.40% (Table 3). Also, the *A. ferrooxidans* suspension turned out to be useful in eliminating hydrogen sulphide. The results of the analysis showed a decrease in the content of this compound of 33.09–33.17% due to the interaction with the bacteria (5- and 10-day incubation periods).

The fourth test was longer (Table 4) and it confirmed the initial results. The most effective bacterial suspension was the *A. thiooxidans* suspension, which caused a significant decrease in the level of H_2S in contaminated process water by 52.51–57.24%, as a result of 20 and 30-day incubation (WT-3 sample). Under the same conditions, similar results were also observed in the case of the *A. ferrooxidans* suspension (WT-4), which reduced the content of the analysed compound by 50.36–54.44%. The third bacterial suspension was *T. thioparus*, which showed a decrease in the level of H_2S of about 20.05–21.50% (WT-1).

Table 4. Changes in biogenic H_2S content in base water samples after adding bacterial suspensions (bacteria representing the genera *Thiobacillus* and *Acidithiobacillus*)

Tabela 4. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody bazowej pod wpływem zastosowania zawiesin bakteryjnych (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension	H_2S content in test samples [mg/dm^3] (base water used in drilling technology)	
		20-day incubation	30-day incubation
WT-1	Thiobacillus thioparus	33.5	33.6
WT-2	Thiobacillus denitrificans	36.8	31.0
WT-3	Acidithiobacillus thiooxidans	19.9	18.3
WT-4	*Acidithiobacillus ferroxidans*	20.8	19.5
K-2 control sample (without any bacterial suspension)	–	41.9	42.8

Table 5. Changes in biogenic H_2S content in formation water samples after adding bacterial suspensions and a nitrate-based inhibitor (bacteria representing the genera *Thiobacillus* and *Acidithiobacillus*)

Tabela 5. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody złożowej pod wpływem zastosowania zawiesin bakteryjnych z inhibitorem azotanowym (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension with 2,000 ppm of the inhibitor (0.2% vol.)	H_2S content in test samples [mg/dm^3] (collective formation water)	
		24-hour incubation	48-hour incubation
WZ-I-1	*Thiobacillus thioparus*	70.9	68.4
WZ-I-2	*Thiobacillus denitrificans*	68.4	69.0
WZ-I-3	Acidithiobacillus thiooxidans	61.2	46.1
WZ-I-4	*Acidithiobacillus ferroxidans*	68.1	67.5
K-3 control sample (without any bacterial suspension or inhibitor)	–	78.0	78.3

Table 6. Research results regarding changes in biogenic H_2S content in formation water samples after application of bacterial suspensions with nitrate-based inhibitor (bacteria representing *Thiobacillus* and *Acidithiobacillus* genera)

Tabela 6. Wyniki badań zmian zawartości biogennego H_2S w próbkach wody złożowej pod wpływem zastosowania zawiesin bakteryjnych z inhibitorem azotanowym (bakterii reprezentujących rodzaje *Thiobacillus* i *Acidithiobacillus*)

Sample designation	Bacterial suspension with 2,000 ppm of the inhibitor (0.2% vol.)	H_2S content in test samples [mg/dm^3] (collective formation water)	
		10-day incubation	30-day incubation
WZ-I-5	Thiobacillus thioparus	40.9	40.2
WZ-I-6	Thiobacillus denitrificans	41.0	40.5
WZ-I-7	Acidithiobacillus thiooxidans	19.7	15.9
WZ-I-8	*Acidithiobacillus ferroxidans*	39.5	38.0
K-4 control sample (without any bacterial suspension or inhibitor)	–	52.0	52.9

Tests results (Tests 5–6)

The last part of the research consisted in experiments based on the combined effect of bacterial suspensions from the genera *Thiobacillus* and *Acidithiobacillus* and a nitrate inhibitor (Table 5). After a short incubation period (24 h), the content of hydrogen sulphide was slightly lower in all the samples tested. From the initial value of 78 mg S^{2-}/dm^3 (control sample K-3), a decrease to 61.2–70.9 mg S^{2-}/dm^3 was observed. On the other hand, the 48-hour incubation showed a satisfactory effect: a 41.12% reduction in H_2S level in the case of the *A. thiooxidans* suspension (WZ-I-4). The remaining suspensions were much less effective.

The last test (Table 6) also showed the most favourable effect when using the *A. thiooxidans* suspension, while the observed decrease in the H_2S content in this fluid after 10 and 30 days of incubation was as high as 61.12–69.94% (WZ-I-7). The remaining three suspensions showed much weaker effects. The results of the analysis of *T. thioparus*, *T. denitrificans*, and *A. ferrooxidans* suspensions were very similar, ranging from 39.5 to 41.0 mg S^{2-}/dm^3 (after a 10-day incubation period) and from 38.0 to 40.5 mg S^{2-}/dm^3 (after a 30-day incubation period); the K-4 control sample ranged from 52.0 to 52.9 mg S^{2-}/dm^3.

Conclusions

1. The methodology used in the research made it possible to assess the possibility of neutralising hydrogen sulphide in the aquatic environment (formation waters and process waters) by using suspensions of bacteria which oxidise sulphur compounds.
2. The bacterial cultures used in the tests displayed different degrees of H_2S neutralisation, which changed during the incubation of the test samples.
3. *Acidithiobaillus thiooxidans* and *A. ferrooxidans* were the most effective species in both aggregate formation water and process water.
4. The test using *A. thiooxidans* demonstrated 32.05–32.25% neutralisation of H_2S after 20 and 30 days of incubation in contaminated formation water. The remaining bacterial suspensions were much less active.
5. The test carried out on process water containing hydrogen sulphide confirmed the previous results. The suspension of *A. thiooxidans* turned out to be the most effective one, causing a significant decrease in the level of H_2S in the contaminated water, amounting to 52.51–57.24% after 20- and 30-day incubation periods, respectively. Under the same conditions, in the case of the *A. ferrooxidans* suspension, a decrease in H_2S content of 50.36–54.44% was observed. The third bacterial suspension was *T. thioparus*, whose activity resulted in a decrease in H_2S of 20.05–21.50%.
6. The experiments based on the joint interaction of bacterial suspensions of the genera *Thiobacillus* and *Acidithiobacillus* and an additional nitrate inhibitor assessed the possibility of combining two methods of action on contaminated formation water. The 48-hour incubation period resulted in a satisfactory reduction of hydrogen sulphide level (41.12%) with the *A. thiooxidans* bacterial suspension. The remaining suspensions were much less effective at the given incubation time (1–2 days).
7. The last test revealed the most favourable effect when the *A. thiooxidans* suspension was used together with the nitrate inhibitor, as the observed decrease in H_2S content in the tested fluid after 10 and 30 days of incubation was very high, amounting to approx. 61–70%. The remaining three suspensions showed much weaker effects. The results obtained from the *T. thioparus*, *T. denitrificans*, and *A. ferrooxidans* suspensions were very similar. They ranged from 39.5 to 41.0 mg S^{2-}/dm^3 (after a 10-day incubation period) and from 38.0 to 40.5 mg S^{2-}/dm^3 (after a 30-day incubation period), with the K-4 control sample ranging from 52.0 to 52.9 mg S^{2-}/dm^3.
8. Based on the tests, it should be concluded that the nitrate agent used in conjunction with an *Acidithiobacillus thiooxidans* bacterial suspension would be a beneficial factor, acting as a biogenic hydrogen sulphide neutralisation agent or as an adjuvant when a biocide or H_2S scavenger (previously used in such environments) degrades.

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