УДК 579.262:546.212

INVESTIGATION OF SULFATE-REDUCING BACTERIA IN PRODUCED WATER FROM THE "NEFT DASLARI" OGED WELLS

E. F. SULTANOV [1, 2]***, N. F. SULTANOVA*** [1]

[1] *Azerbaijan State Oil and Industry University, Baku, Azerbaijan*

[2] *OilGasScientificResearchProject Institute of SOCAR, Baku, Azerbaijan*

Abstract. *Sulfate-reducing bacteria (SRB) are recognized as key contributors to microbiologically influenced corrosion (MIC), a major challenge in oil and gas industries. These anaerobic microorganisms are capable of reducing sulfate ions (SO_4^{2-}) to hydrogen sulfide (H_2S), thereby accelerating electrochemical corrosion processes on metallic surfaces. Moreover, SRB activity promotes biofilm formation, which further enhances the persistence and severity of corrosion. In this study, the presence of sulfate-reducing bacteria were investigated in produced water samples collected from the production wells of the "Neft Daslari" Oil and Gas Extraction Department. The obtained results contribute to understanding the microbial diversity and corrosion potential of subsurface environments associated with offshore oil extraction.*

Keywords: *anaerobic microorganisms, sulfate-reducing bacteria, microbiologically influenced corrosion, biofilm, microscopy.*

Introduction. Sulfate-reducing microorganisms (SRM) constitute a physiologically diverse group of prokaryotes, including representatives from both the *Bacteria* and *Archaea* domains, that play an essential role in the global sulfur and carbon cycles (Pham et al., 2009). To date, more than 220 species across 60 genera have been identified. Despite their metabolic diversity, all SRB share a common physiological feature–the ability to use sulfate (SO_4^{2-}) or other oxidized sulfur compounds as terminal electron acceptors during the oxidation of organic matter under strictly anaerobic conditions.

A wide range of substrates can serve as electron donors in their metabolism, including molecular hydrogen (H_2), lactate, acetate, pyruvate, malate, ethanol, propanol, and butanol (Sette et al., 2007). Some SRB species, such as those from the genera *Desulfovibrio* and *Desulfobacterium*, are also capable of utilizing nitrate as an alternative terminal electron acceptor. Depending on the bacterial strain, organic substrates may be fully oxidized to carbon dioxide (e.g., *Desulfomicrobium*) or partially to acetate (e.g., *Desulfovibrio*). This respiratory pathway, known as dissimilatory sulfate reduction, is unique to SRB and is not performed by any other microorganisms. During this process, only a small fraction of reduced sulfur is assimilated by the cell, while the majority is released as hydrogen sulfide (H_2S) into the surrounding environment (Peng et al., 1994).

Hydrogen sulfide is a highly reactive compound that induces the formation of iron sulfide (FeS) precipitates on metallic surfaces (Figure 1). This accelerates the deterioration of steel infrastructure through electrochemical reactions and undermines the structural integrity of pipelines, tanks, and offshore platforms (Okabe et al., 1994). The formation of SRB-associated biofilms further protects bacterial communities from environmental stress and enhances corrosion persistence (Kumaraswamy et al., 2011). Consequently, SRB activity presents both operational and environmental challenges in oil and gas industries, leading to production losses, safety hazards, and increased maintenance costs.

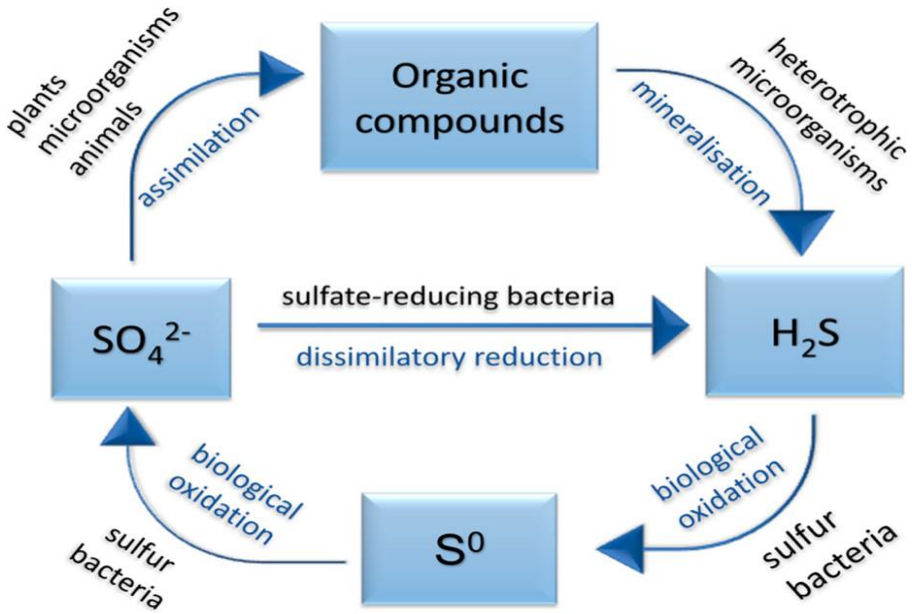

Figure 1. – Schematic representation of the sulfur cycle involving sulfate-reducing microorganisms

The main objective of this study was to identify and quantify sulfate-reducing bacteria in produced water samples collected from wells at the “Neft Daslari” OGED.

I. Materials and methods. Produced water samples were collected from several wells at the “Neft Daşları” Oil and Gas Extraction Department. Microbiological analyses were carried out using selective culture media appropriate for each microbial group. The enumeration of sulfate-reducing bacteria was performed following the NACE TMO194-2014 standard protocol ("Field Monitoring of Bacterial Growth in Oil and Gas Systems"). For each sample, 1 mL of produced water was aseptically transferred into a sterile glass vial containing 9 mL of Postgate’s medium. The mixture was homogenized for 5 minutes to obtain the first serial dilution. Further serial dilutions were prepared at ratios of 1:100, 1:10,000, and higher, as required. All inoculated samples were incubated anaerobically at 32 °C for 15 days. Media was autoclaved at 121 °C for 20 min. The presence of SRB was determined based on visible changes in the medium, such as blackening due to the formation of iron sulfide precipitates. The bacterial concentration (cells/mL) was calculated using the following equation: $X=10n\times10/V$, where n is the dilution factor at which bacterial growth was last observed, and V is the volume (mL) of the inoculated sample.

The glass bottles were incubated for 7 days at 32 °C. Blackening of the bottle meant a positive growth for SRB. This step was repeated at least 10 times to obtain SRB. Microscopic observations were conducted to confirm the presence of characteristic SRB morphotypes under a compound light microscope (model: Olympus CX43, Japan), and data were interpreted in relation to their possible impact on corrosion in oilfield systems.

The dynamics of sulfide formation by SRB were monitored over seven days, and sulfide concentrations were quantified using the iodometric method described by APHA (1989).

II. Results and discussion. Microbiological analyses of the produced waters revealed detectable populations of sulfate-reducing bacteria, with cell densities ranging from 10^4 to 10^6 cells/mL, depending on the sampling location. Chemical characterization of the produced waters (data not shown) suggested favorable conditions for SRB proliferation, including high salinity, sulfate availability, and reducing redox potential. Thioglycolic acid and ascorbic acid were incorporated into media formulations to enhance their overall reducing capacity (Table 1).

Table 1. – Chemical composition of the modified Postgate B medium for SRB growth

Chemical Ingredient	Chemical Formula	Concentration (g/L)	Remarks / Purpose
Potassium dihydrogen phosphate	KH_2PO_4	0.5	Source of phosphate buffer; maintains pH stability
Ammonium chloride	NH_4Cl	1.0	Nitrogen source for bacterial growth
Sodium sulfate	Na_2SO_4	1.0	Primary electron acceptor for SRB metabolism
Calcium chloride hexahydrate	$CaCl_2 \cdot 6H_2O$	0.1	Supplies calcium ions; stabilizes cell walls
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	2.0	Source of magnesium for enzymatic activity
Sodium lactate (60–70%)	$CH_3CH(OH)COONa$	5 mL	Main carbon and energy source for SRB
Yeast extract	–	1.0	Provides vitamins, amino acids, and growth factors
Ascorbic acid (Vitamin C)	$C_6H_8O_6$	0.1	Reducing agent; maintains anaerobic conditions
Thioglycolic acid	$HSCH_2COOH$	0.1	Reducing agent; prevents oxidation of sulfides
Ferrous sulfate heptahydrate	$FeSO_4 \cdot 7H_2O$	0.5	Provides ferrous ions; supports enzyme activation
Sodium chloride	NaCl	26	Maintains osmotic balance (simulates saline conditions)
Sea water	–	500 mL	Provides trace minerals and ions
Distilled water	–	500 mL	Solvent for medium preparation
pH (adjusted to)	–	7.0 – 7.5	Optimal for SRB growth and sulfate reduction

Postgate Medium B is a versatile formulation designed for the isolation, detection, and cultivation of sulfate-reducing bacteria such as *Desulfovibrio* and *Desulfotomaculum* species. Most of the medium's components can be prepared in advance and stored as stock solutions; however, the reducing agents–thioglycolic acid and ascorbic acid–should be freshly added, and the pH adjusted immediately prior to autoclaving. These compounds are optional when using active and recently subcultured inocula but are essential for maintaining strong reducing conditions in older or less active cultures. The medium should be utilized promptly after cooling, as the reducing agents are unstable and gradually oxidize upon exposure to air at neutral pH, often producing a temporary purple coloration. The characteristic precipitate formed in Postgate Medium B provides a suitable environment for tactophilic strains, supporting robust growth. This medium is also suitable for long-term maintenance of SRB strains under anaerobic conditions. Overall, successful cultivation of pure SRB cultures primarily depends on maintaining a strictly anaerobic environment and a stable redox balance. Members of the genus *Desulfotomaculum* were identified as dominant representatives in the studied samples. These endospore-forming SRB are typically found in deep subsurface environments, sediments, and produced waters. The genus *Desulfotomaculum* belongs to the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales*, and family *Peptococcaceae*. These Gram-positive, motile rods range from 0.3–2.5 × 2.5–15 µm in size and may occur singly or in pairs. Their spores, which

are oval or spherical and located terminally or centrally within the cells, often cause noticeable swelling of the cell body.

The sulfide production by SRB was monitored over a 7-day incubation period to evaluate the time course of SRB metabolic activity in the Postgate (B) medium. This medium is specifically designed to promote SRB growth and activity due to its balanced nutrient composition and the presence of sulfate as the terminal electron acceptor. The experiment was conducted in triplicate using sterile 250 mL serum bottles containing 100 mL of Postgate (B) medium inoculated with the SRB culture under anaerobic conditions. The cultures were incubated at 32 ± 2 °C for 7 days. At 24-hour intervals, samples were aseptically withdrawn to quantify the produced sulfide, reflecting the metabolic reduction of sulfate (Figure 2).

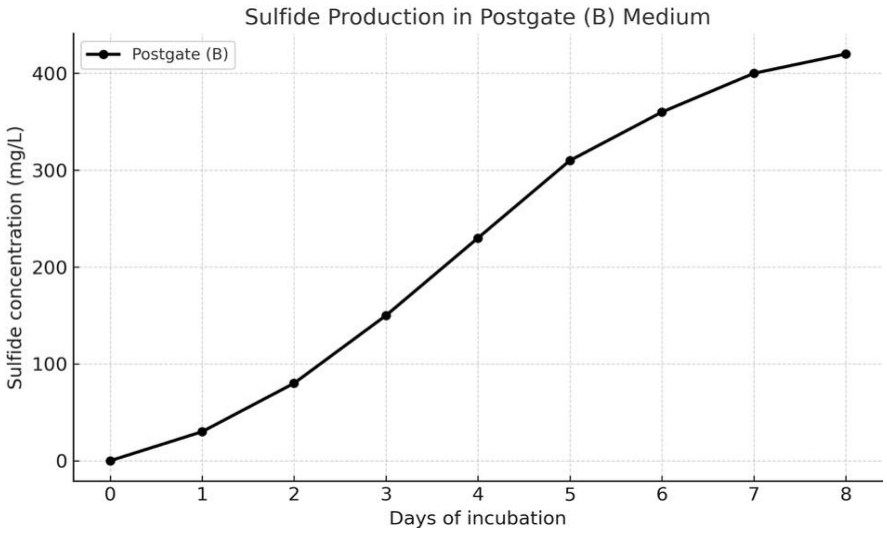

Figure 2. – Time course of sulfide production by SRB during seven days incubation at 32 °C in Postgate (B) culture media

The sulfide concentration was determined iodometrically following the standard procedure of the American Public Health Association (APHA, 1989). In this method, sulfide ions react stoichiometrically with iodine in an acidic medium, allowing quantitative determination of sulfide through titration. Briefly, a measured aliquot of the culture supernatant was acidified with 6 N HCl to release dissolved sulfide as H_2S gas, which was immediately trapped and titrated with 0.025 N iodine solution using starch as an indicator. The end point was observed as a color change from colorless to pale blue, indicating the complete oxidation of sulfide. The measured sulfide concentrations (mg L^{-1}) were plotted as a function of incubation time (days), showing a gradual increase in sulfide accumulation corresponding to enhanced SRB activity (Figure 2). Maximum sulfide production was observed on the 7^{th} day, reaching approximately 400 mg L^{-1}, indicating the active reduction of sulfate to sulfide under favorable anaerobic conditions.

Desulfotomaculum species exhibit remarkable physiological versatility, thriving under mesophilic (30–37°C) or thermophilic (50–65°C) conditions and adapting to fluctuating redox environments. Their ability to form spores enables them to persist under unfavorable environmental conditions, such as oxygen exposure or nutrient limitation–making them particularly resilient in oilfield ecosystems.

SRB have been reported in a wide variety of habitats, including freshwater and marine sediments, geothermal springs, hypersaline waters, and even within animal gastrointestinal tracts. Their widespread occurrence and adaptability underscore their ecological significance and potential to influence corrosion processes in both natural and industrial systems.

Conclusion. The detection of sulfate-reducing bacteria in produced waters from the "Neft Daşları" field confirms the microbial contribution to corrosion processes within oil production infrastructure. The metabolic activity of SRB, particularly the production of hydrogen sulfide (H_2S), poses serious operational risks by accelerating metal corrosion, reducing equipment lifespan, and increasing maintenance costs. Moreover, H_2S is a toxic and environmentally hazardous gas that can compromise workplace safety and contribute to environmental pollution.

Comprehensive monitoring and control of SRB populations are therefore essential for the sustainable management of oilfield operations. Understanding their ecology and metabolic potential will facilitate the development of targeted biocorrosion mitigation strategies, thereby improving the safety, efficiency, and environmental sustainability of petroleum extraction activities.

REFERENCES

1. American Public Health Association (APHA). (1989). Identification of iron and sulfur bacteria. In Standard methods for the examination of water and wastewater (14th ed.). APHA, Inc.
2. Kumaraswamy, R., Ebert, S., Gray, M. R., & others. (2011). Molecular and cultivation-based analyses of microbial communities in oil field water and in microcosms amended with nitrate to control H_2S production. Applied Microbiology and Biotechnology, 89(6), 2027–2038.
3. Okabe, S., Nielsen, P. H., Jones, W. J., & Characklis, W. G. (1995). Sulfide product inhibition of Desulfovibrio desulfuricans in batch and continuous cultures. Water Research, 29(1), 175–187.
4. Peng, C. G., Park, C. G., Park, J. K., & Patenaude, R. W. (1994). Statistical basis of classification of microbial influenced corrosion in freshwater systems. Water Research, 28(4), 951–959.
5. Pham, V. D., Hnatow, L. L., Zhang, S., Fallon, R. D., Jackson, S. C., Tomb, J. F., DeLong, E. F., & Keeler, S. J. (2009). Characterizing microbial diversity in production water from an Alaskan mesothermic petroleum reservoir with two independent molecular methods. Environmental Microbiology, 11(1), 176–187.
6. Sette, L. D., Simioni, K. C., Vasconcellos, S. P., Dussan, L. J., Neto, E. V., & Oliveira, V. M. (2007). Analysis of the composition of bacterial communities in oil reservoirs from a southern offshore Brazilian basin. Antonie van Leeuwenhoek, 91(3), 253–266.