

Use of Novel, Rapid Enzyme Based Assays to Detect the Presence of Sulfate-Reducing Bacteria in Oil and Gas Produced Water

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ABSTRACT

Biocides are commonly used to manage bacteria populations on oil and gas production facilities. Each biocide active ingredient, formulation, mode of application, and dose rate will produce different effects on bacteria populations. The most effective way to accurately ascertain the anticipated effect of biocides is to conduct bacteria population testing. As sulfate-reducing bacteria (SRB) are known to sour production and contribute to both chemical and microbial induced corrosion, oil and gas production operators routinely conduct testing to assess SRB populations. Effective biocide management requires accurate knowledge of both total bacterial load and the presence of SRB. This paper reports on the design, application and interpretation of two separate enzyme-based assays. One assay determines total bacteria load, while the other detects the presence of SRB. The enzymatic assays react with bacteria enzymes, producing dye that is proportional to the magnitude of the bacteria population after a period of exposure. The assays deliver results within one day. They are compact, portable, and rely on non-hazardous, non-toxic materials with no special incubation or refrigeration required. By combining these results, an in-depth bacteria risk assessment can be conducted. The assays can also be used before and after biocide application to determine if treatment meets bacteria management goals.

Key words: Microbial Induced Corrosion (MIC), bacteria, asset souring, sulfate-reducing bacteria (SRB), enzyme assay, bacteria management, hydrogen sulfide (H₂S), biocide

INTRODUCTION

Biocides are commonly used for bacteria management in oil and gas production facilities. Effective oil and gas biocide application management should be optimized in response to risk assessments. Optimizing biocide application requires rapid and accurate knowledge of the total bacterial load, as well as the detection of sulfate-reducing bacteria (SRB) due to the unique risks they present. Each biocide active ingredient, formulation, mode of application, and dose rate will produce different effects on bacterial populations. This study reports on combining an established enzyme-based assay that determines the total living bacteria population and the design, application, and interpretation of a second assay that detects the presence of SRB.

SRB are a significant concern for oil and gas operators due to their role in souring production and contributing to microbial induced corrosion (MIC).¹ These anaerobic bacteria metabolize sulfates to produce hydrogen sulfide gas (H₂S), which accelerates chemical corrosion, poses safety and environmental hazards, and results in financial losses.^{2,3} SRB can also contribute to the formation of biofilms that create favorable environments for MIC by accelerating its electrochemical processes.³

SRB populations are routinely assessed by collecting and processing water samples within 24 hours⁴ to ensure they accurately represent site conditions. Effective biocide applications to mitigate SRB activity and MIC require a comprehensive understanding of the system, including site history, water injection rates, modes of injection, and corrosion monitoring programs. Additionally, measuring the total bacterial load and identifying problematic bacteria are critical for designing targeted treatment strategies. This information ensures the selection of appropriate biocides for the ever-changing conditions in oilfield applications.

The oil and gas industry uses bacteria detection technologies including culture techniques, metagenomics, and metabolism assays. The AMPP standard for the serum serial dilution procedure can be prone to inaccuracies inherent to bacteria culture techniques.⁵ It also generates a large waste stream that contains hazardous components. When conducted by AMPP standards, serial dilution culture testing requires incubation and refrigerated storage. A single test requires advanced testing and planning to ensure salinity matching to sample water, 18 glass culture bottles, needles, syringes, alcohol wipes, and up to a 28-day incubation period to perform the test.^{5,6} Nucleic acid testing (qPCR, mRNA, NGS) gather information about the microbial makeup of a sample, determining species presence.^{6,7} Although some mobile metagenomic kits are available⁸, samples are often sent to commercial laboratories, requiring processing times of several weeks. This delay in obtaining results can significantly hinder timely treatment strategies. Measurements of cellular ATP are used as a proxy for direct bacteria population measurement. However, ATP testing does not identify bacteria by species and does not offer direct or indirect bacteria population enumeration.⁹ Because of these limitations, a need for faster and more accurate methods for bacteria testing are apparent.

This study evaluated the value of combining two onsite enzyme-based bacterial assays. The first assay measures the total bacterial content in water samples, while the second detects the presence of SRBs. The assay used to determine total bacteria load was introduced to oil and gas in 2019.¹⁰ This method has undergone extensive testing and calibration to prove accuracy, reproducibility, repeatability, and compatibility across all water-based fluid applications. Prior to field management application, over 20,000 individual tests were conducted to calibrate the technique, explore potential interferences, and determine method sensitivity.¹⁰ This enzyme assay chemistry targets bacterial populations but does not identify specific microbial species. The SRB specific assay is relatively new to the oil and gas industry, but provides onsite species-specific testing, enhancing the representation of the samples.

METHODS

Total Population Testing

The total bacteria population assay includes a normalization calculation that adjusts a sample reading value for, an analytical blank, the volume of samples filtered, the exposure time to the substrate complex and the temperature of the sample. The standardized value is then converted using a propriety algorithm developed from previous method calibrations to either colony forming units / ml (CFU/ml), or equivalent serial dilution bottle turns using a mobile application or computer program as shown in Figure 1. Each batch of tests includes a set of control bacteria samples to ensure the method is performed correctly. Typically, it takes 30 minutes to analyze a water sample but 10 – 20 samples can be analyzed concurrently.¹⁰

Figure 1: Schematic of Monitoring Method and Conversion Software (Mobile Application)¹⁰

The method does not contain sharps or require incubation and comes with all components needed for use. To prolong the shelf life of the enzyme substrate solution, refrigeration is recommended, as the substrate will begin to degrade in > ambient temperatures over time. This method provides valuable insight into the overall bacterial risk within a system and can be used to assess biocide efficacy through kill studies.

Novel Enzyme Based SRB Detection Method

SRB detection in this novel assay relies on sulfite reduction, a key metabolic process unique to SRB. The test targets the SRB-specific enzyme dissimilatory sulfite reductase, producing H_2S as a byproduct seen in Figure 2.^{2,3,11} The detection system uses a reagent bound to a dye molecule that is released upon interaction with the enzyme. This release creates a visible correlation between the amount of SRB present and the observable color of the sample. The test is designed as a fluorometric assay, where the released dye produces a visible color change and fluorescence. When illuminated with low-energy blue light, samples containing SRB emit a greenish yellow glow.

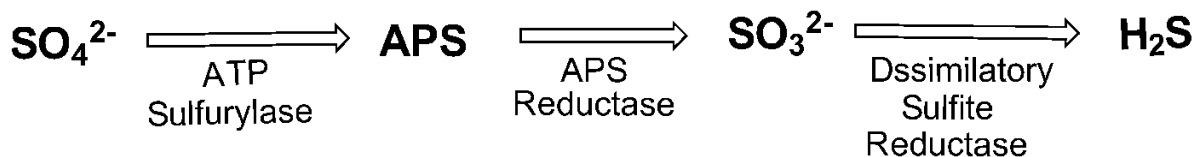


Figure 2: Sulfate Reducing Bacteria Metabolism Reaction¹¹

Potential false positives caused by the presence of mercaptans or interactions with other bacterial species, are addressed by employing a two-part assay detection system.¹¹ The first assay detects the bacterial enzyme known to be present in SRBs, while the second assay confirms the presence of H_2S . Since SRB produces H_2S , both assays must return positive results for the sample to be confirmed as SRB-positive. The SRB assay produces a greenish yellow glow under blue light, while the H_2S assay results in a color change from clear to yellow in ambient lighting. This dual-detection approach ensures accurate results by negating false positives and providing reliable confirmation of SRB presence.

Visual comparators with established minimum detection limits provide standard references for confirmation. SRB presence is verified when both SRB and H₂S assays show observable color development. For H₂S, the comparator cuvette is yellow, representing a concentration of 2 ppm.¹¹ A positive result occurs when the sample turns yellow with a color intensity matching or exceeding the provided prefilled comparator. For SRB, the comparator is a clear cuvette that glows greenish yellow under light emitted by a handheld device included in the test kit. This glow corresponds to approximately 10³ bacteria/ml (3 log).¹¹ A positive SRB result is indicated when the sample glows with an intensity equal to or greater than the comparator. Example positive results are presented in Figure 3. The handheld device, or light box, uses low-energy light to facilitate detection.

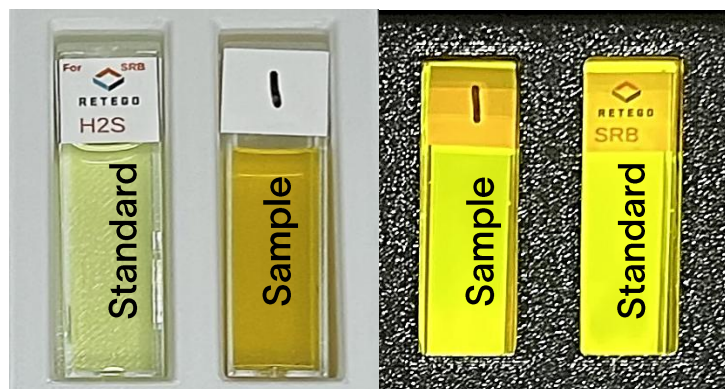


Figure 3: Example Positive H₂S Assay (left) and SRB Assay (right)

The test was created as a field ready method for SRB testing and was designed to contain all components needed to conduct the assay. (See Figure 4). This novel approach uses dehydrated reagents seen as a white or yellow powder, packaged in individual capped cuvettes to aid in rapid dissolution to reduce introduction of oxygen in the sample that can occur with vigorous mixing.¹¹ SRB are obligate anaerobes, either dying or damaged in the presence of oxygen.¹¹ This characteristic often results in delayed reaction kinetics, as SRB must first deplete oxygen in their environment before engaging in metabolic activity, which can take hours, days, or even weeks.^{3,11} To overcome this limitation, the assay incorporates an oxygen scavenger, enabling faster reaction kinetics and quicker SRB identification.¹¹



Figure 4: Rapid Onsite SRB Detection Kit

The SRB test uses water-based fluid samples collected from various field sources, including fresh water, seawater, and produced water. Using a single-use transfer pipette, the collected water samples are added to cuvettes, already prefilled with dry reagents, which are then gently inverted to ensure thorough dissolution of the reagents. The cuvettes are left to sit for a maximum of 24 hours, allowing sufficient time

for the bacterial enzymes to interact with the assay, trigger a reaction, and produce fluorescence.¹¹ The H₂S assay results can be confirmed within 8 hours, with checks performed 30 minutes after introducing the sample and periodically up to the 8-hour cutoff. Similarly, SRB assay results are confirmed within 24 hours, with observations conducted every 4 hours up to the 24-hours. A color change can be observed immediately or at any time over the 24-hour time frame. It is recommended to record any observations to aid with the analysis of the data. The intensity and timing of the color change can help estimate the bacterial population in the sample. This rapid testing method delivers results in less than a day, facilitating both detection and confirmation of H₂S and SRB presence in water samples.

RESULTS

Prior to field testing, a study was conducted to validate the novel SRB testing technology against the NACE standard techniques used for SRB detection. Field testing was conducted at multiple field locations and field sites to further evaluate the new SRB test against total bacteria population.

SRB Test Validation Study

SRB cultures were grown in Modified Postgate's B media (MPB)¹², which is the NACE TM0194 standard. A master broth with a known high bacterial concentration was prepared by a third-party lab and inoculated into serum serial dilution vials according to the standard. Samples for both the SRB and total bacteria population tests were taken directly from these dilution vials. To monitor bacterial activity over time, a set of samples were tested at intervals of 1, 3, 5, and 7 days, continuing until all the serial dilution vials displayed a color change. A total of 18 bottles were prepared for each testing day, with one set removed from incubation for analysis on the specified testing day.

By day 7, all media bottles exhibited a color change from clear to black, indicating the presence of sulfides and confirming SRB-positive results. Because the media bottles changed color no further testing was conducted after day 7. By the end of the testing period, the media bottles represented a bacterial concentration of approximately 100,000–1,000,000 bacteria/ml (5-6-log presence). For each SRB test, approximately 3 ml sample was used, while 5 ml was allocated for total bacteria population testing. Observations of H₂S results were recorded immediately and again after 30 minutes. For SRB, observations were recorded immediately with additional observations made at 4, 8, 12, and 24 hours to evaluate any progression of SRB activity. Total population testing was conducted at time intervals of 0, 10, 20, and 30 minutes on each day and averaged for analysis.

Table 1
Validation Study Results

	Day 1			Day 3			Day 5			Day 7		
Vial	Total Living Bacteria/ml	New SRB Assay	New H ₂ S Assay	Total Living Bacteria/ml	New SRB Assay	New H ₂ S Assay	Total Living Bacteria/ml	New SRB Assay	New H ₂ S Assay	Total Living Bacteria/ml	New SRB Assay	New H ₂ S Assay
1	391,000	+	+	332,000	+	+	1,065,750	+	+	376,750	+	+
2	344,000	+	+	406,500	+	+	498,500	+	+	480,500	+	+
3	351,000	+	+	310,750	+	+	457,250	+	+	478,000	+	+
4	350,000	+	+	299,000	+	+	348,500	+	+	328,250	+	+
5	348,000	+	+	257,500	+	+	333,250	+	+	332,500	+	+

Table 1 results yielded comparable results of the new SRB detection test evaluated against serum serial dilution. Over the 7-day testing period, the SRB test consistently detected SRB concentrations in all serial dilutions of the dilution bottles. Fluorescence observations for SRB at all testing times and on each testing day were positive. The H₂S assay also yielded positive results at all periods of collection. It was observed that the cuvettes showed a positive color change immediately upon introduction for the media, which means the samples contained more than 2 ppm of H₂S, and confirms any positive SRB results. The total bacteria load test consistently resulted in around 300,000 bacteria/ml or 5 logs for the bottle turn equivalent, further supporting the bottle turns and SRB detection results.

Field Case Study Results

Produced water samples were collected from 9 mature conventional oil wells. A mixture of wells was selected that had a testing history, some positive and some negative for SRB bacteria with the standard serum serial dilution method. The produced water samples were tested using the techniques described in the methods.

Table 2
Field Study Results

Well Code	Total Living Bacteria/ml	SRB Serum Serial Dilution	New SRB Assay/ hours to results	New H ₂ S Assay/ hours to result
A	10	0	Negative/24	Negative
B	0	0	Negative/24	Negative
C	0	0	Negative/24	Negative
D	4000	3	Positive/8	Positive/1
E	20000	4	Positive /4	Positive/1
F	400	1	Positive/24	Positive/6
G	8300	3	Positive/12	Positive/3
H	0	0	Negative	Negative
I	0	0	Negative	Negative
J	70000	4	Positive/1	Positive/ <1
CONTROLS				
Potable Water	200	0	Negative	Negative
Bottled Water	0	0	Negative	Negative

Results from Table 2 compare the two metabolism techniques. A positive relationship can be observed between wells D, E, F, G, and J found to have high bacteria contamination levels and positive test results from the SRB and H₂S assays. Additionally, it can be noted that the hours to results for the SRB test resulted in an observable visual change in less time for samples shown to have a higher bacterial count and vice versa. No false positives were observed from the SRB test, and all SRB-positive results were confirmed with a H₂S-positive result.

DISCUSSION

To evaluate the efficacy and ease of use in oil and gas bacteria management, two enzyme-based assay tests were used during a field bacteria survey. One assay measured the total bacterial population, while the other detected the presence of SRB in the sample. Prior to field use, the novel SRB detection assay

was validated using standard SRB serum serial dilution selective culture to confirm its ability to detect SRB. The SRB assay identified SRB presence before the dilution bottles exhibited significant color changes or detectable sulfide production. The study demonstrated that the SRB test was able to detect SRB as low as 3 log CFU/ml and up to 6 log CFU/ml. Results from the total bacteria test further confirmed high microbial concentrations, showing 5–6 log bacterial presence in the sample and supporting the SRB findings. The field study aimed to test the field suitability of the new SRB test and how it responded next to total bacteria population testing. In the field the SRB results were nearly identical to standard SRB culture methods. The findings support the new SRB test's ability to detect the presence of SRB, with results that show a positive indication for samples with > 3 log of bacteria and SRB cultures and negative results from wells with low (< 3 log) bacterial risk.

By combining the two methods, field operators can gain a more comprehensive understanding of the microbiological risks present in their systems. Total bacterial load testing provides insight into the overall level of contamination, while SRB-specific testing identifies the presence and activity of one of the most problematic bacteria present in oilfield. The new SRB detection kit offers unique features desirable for field oil and gas testing. It is lightweight, contains non-hazardous, non-toxic components that can be disposed of as standard waste, and does not utilize sharps or glass. The kit contains pre-filled cuvettes with dried reagents, sealed with airtight caps for easy storage, transport, and use. Results can be visually confirmed using the provided standard comparisons, eliminating the need for complex detection electronics. Both the SRB detection and total bacteria population methods are portable, cost-effective, and designed for practical use in field conditions. These ready-to-use kits use enzyme assay chemistry to deliver rapid results (within 1 day), designed for convenience and practicality enhancing their oilfield applications. Together, these methods enable more informed decisions regarding biocide selection, dosing, and application, reducing the risks associated with MIC, reservoir souring, and H₂S production.

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REFERENCES

1. Y. N. Elshahed, M.S., and M.J. McInerney, "Microbial Processes in Oil Fields: Culprits, Problems, and Opportunities," *Advances in Applied Microbiology*, vol. 66, pp. 141–251 (2009), doi: 10.1016/S0065-2164(08)00806-X.
2. T.T.T. Tran, K. Kannoorpatti, A. Padovan, and S. Thennadil, "Sulphate-Reducing Bacteria's Response to Extreme pH Environments and the Effect of Their Activities on Microbial Corrosion," *Applied Sciences*, vol. 11, no. 2201 (2021), doi: <https://doi.org/10.3390/app11052201>.
3. G. Muyzer and A.J.M. Stams, "The Ecology and Biotechnology of Sulphate-Reducing Bacteria," *Nature Reviews Microbiology*, vol. 6, issue 6, pp. 441–454 (2008), doi: <https://doi.org/10.1038/nrmicro1892>.
4. U.S. EPA, "Quick Guide to Drinking Water Sample Collection," Second Edition, Update September 2016.
5. NACE TM0194 (2014), "Field Monitoring of Bacterial Growth in Oil and Gas Systems", (Houston, TX: NACE)
6. R. de Paula and V. Keasler, "Molecular Techniques for Onsite Monitoring of Microbial Populations in Oilfields: Overview, Perspective, and Evaluation." Available at: <http://onepetro.org/NACECORR/proceedings-pdf/CORR16/CORR16/1346105/nace-2016-7698.pdf>.

7. R.M. de Paula, L. Gieg, K. Duncan, N. Tsesmetzis, R. Eckert, and T. Lund Skovhus, "Time to Agree: The Efforts to Standardize Molecular Microbiological Methods (MMM) for Detection of Microorganisms in Natural and Engineered Systems." Available at: <http://onepetro.org/NACECORR/proceedings-pdf/CORR21/CORR21/D111S046R006/2447179/nace-2021-16986.pdf/1>.
8. KRAKEN™. (n.d.). Rapid qPCR microbial monitoring in oil & gas. KRAKEN™. Retrieved September 16, 2025, from <https://krakensense.com/oil-gas>
9. D.G. Bennet, "Oilfield Microbiology: Detection Techniques Used in Monitoring Problematic Microorganisms Such as Sulphate-Reducing Bacteria (SRB)," OTC-26788-MS, 2016.
10. J. Fajt, A. Murphy, and A. Jenkins, "Development and Field Application of a New Bacteria Monitoring Technique," NACE 2019-13158, 2019.
11. C. Lloyd and A. Duncan, "System and Kit for Rapid Multimodal Detection of Anaerobic Sulfate-Reducing Bacteria via Integrative Spectral Comparison" (U.S. Patent Application US2025/0230482A1, January 9, 2025, and PCT WO2025/151600A1, July 17, 2025).
12. Biotechnology Solutions. Modified Postgate's B Media (MPB). Retrieved [September 16,2025], from <https://bts-biotechnology.com/modified-postgates-b-media-mpb/>