



Mitigating Sulfate-Reducing Bacteria in Produced Water: Performance of MB-X Biocide Assessed by Sulfate-Reducing Bacteria Biological Activity Reaction Test at Regional 1 Sumatera

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ABSTRACT - Sulfate-reducing bacteria (SRB) are major drivers of microbiologically influenced corrosion (MIC) and reservoir souring in waterflooding operations, particularly in mature onshore assets with high produced-water volumes. This is showing the need for effective control of SRB to mitigate potential risks and maintain the integrity of waterflooding operations. Therefore, this study is aiming to evaluate the efficacy of the biocide MB-X at multiple doses (50-200 ppm) in suppressing SRB activity and population using the SRB-BART (Biological Activity Reaction Test). The experiment is being conducted by collecting produced-water samples from the Outlet WT at Field MX, followed by daily monitoring of SRB activity to determine the reaction day. The data obtained are being converted into population estimates (CFU/mL) according to the commercial kit guidance: Day-3 = 1.15×10^5 CFU/mL; Day-4 = 2.7×10^4 CFU/mL; Day-10 = 5 CFU/mL; Day-11 < 1 CFU/mL. Aggressiveness is being classified according to the kit scoring, namely Aggressive, Not Aggressive, and Negative. The results are showing that the control (blank) is showing a BB-type SRB consortium (anaerobe-dominant), with 1.15×10^5 CFU/mL being classified as Aggressive. MB-X 50 ppm is reducing the population to 2.7×10^4 CFU/mL, but the response is remaining Aggressive. In comparison, MB-X 100 ppm is suppressing counts to 5 CFU/mL (Not Aggressive), while MB-X 200 ppm is yielding a Negative response, showing undetected SRB activity. These results are indicating that MB-X at ≥ 100 ppm is effectively controlling SRB under test conditions, with 200 ppm achieving apparent eradication as detected by SRB-BART. However, interpretation of the statistical outcomes is being constrained by the limited number of treatment replicates, suggesting that the results should be considered a preliminary site-specific evaluation of MB-X performance. Consequently, future studies are being recommended to conduct field verification through replication, matrix controls, and in-situ corrosion monitoring to confirm performance durability.

Keywords: sulfate-reducing bacteria, MIC, produced water, biocide MB-X, SRB-BART, mature onshore asset.

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1. Introduction

Sulfate-reducing bacteria (SRB) are a group of anaerobic microorganisms that use sulfate as a terminal electron acceptor and produce hydrogen sulfide (H₂S). In the oil and gas industry, SRB contribute significantly to reservoir souring and microbiologically influenced corrosion (MIC) of carbon-steel pipelines, pressure vessels, and processing equipment, thereby increasing leakage risk and asset maintenance costs (OPEX) (Spark et al., 2020). The MIC is typically characterized by the formation of ferrous sulfide (FeS) deposits and progressive pitting corrosion. Previous studies in upstream operations have reported premature pipeline failures associated with SRB activity and the accumulation of H₂S within biofilms (Gerrit Voordouw et al., 2017; Udowo et al., 2023).

In produced or treated water systems and waterflooding operations, SRB commonly persist as constituents of biofilms adhered to metal surfaces, showing high resistance to chemical treatments and conventional microbiological monitoring (Okpala et al., 2026). Consequently, mitigation strategies must address dissolved H₂S and the underlying bioburden of these microorganisms to prevent microbial resurgence and the continuation of corrosion processes (PureLine 2025). Experimental evidence shows that SRB biofilms require higher biocide doses than planktonic cells to achieve significant suppression of sulfidogenesis, determining a minimum effective dose (MED), a critical component of control programs (Fajt 2023).

For rapid detection and semi-quantitative monitoring, the SRB-BART (Biological Activity Reaction Test) is widely used. This method uses blackening intensity, BB/BT dominance patterns, and days-to-reaction as indicators of SRB population and aggressiveness (Hach Company 2016). The reaction time provides a practical basis for estimating SRB population levels, with official guidelines connecting reaction-day values to estimated populations (CFU/mL). For example, a Day-1 reaction corresponds to 2.2×10^6 CFU/mL, Day-3 to approximately 1.15×10^5 CFU/mL, Day-4 to 2.7×10^4 CFU/mL, while Day-10 (~ 5 CFU/mL) and Day-11 (< 1 CFU/mL) show very low populations and “Not Aggressive” behavior (Tm 2014). In addition to providing presence/absence and dominant-pattern outcomes, SRB-BART is practical for field monitoring due to the lack of specialized culturing facilities and can be observed daily for ≥ 10 –15 days as recommended in technical advisories.

Chemical SRB control is commonly achieved through biocide application using shock (intermittent) or continuous dosing schemes. The effectiveness of these treatments depends on several site-specific factors, including microbiome composition, biofilm matrix characteristics, and water chemistry such as oxidant consumption by sulfide/thiosulfate. Therefore, dose-response testing using site-specific matrices is essential before full implementation (Okpala et al., 2026). Laboratory and field studies have shown that agents such as ADBAC, THPS, and glutaraldehyde can be effective at tens to hundreds of ppm, although optimal doses vary across locations, and established biofilms require higher doses to eliminate sulfidogenic activity (Fajt 2023). Establishing the minimum effective dose (MED) from laboratory and field data, followed by in-situ performance verification using methods such as corrosion coupons, is critical for balancing performance, cost, and risk (Xu et al., 2024).

Recent literature has reported that SRB embedded in biofilms are more challenging to eradicate than planktonic cells and require sufficient biocide penetration, assisted by bio-dispersants or biocide enhancers. Furthermore, combination strategies can reduce required biocide doses for effective control. For example, THPS combined with biofilm-disrupting peptides has been shown to reduce pitting and mass loss in 13Cr steel exposed to highly corrosive SRB. Nitrite pretreatment that lowers cellular metabolic energy can enhance the efficacy of glutaraldehyde, allowing for dose reduction. Due to the diversity and site-specific nature of oilfield microbiomes, dose determination based on local datasets remains best practice (Bødtker et al., 2008; Javier Vilcaez et al., 2006; Xu et al., 2024).

Despite the widespread application of biocide programs, two practice gaps persist in many water-injection facilities. First, programs often rely on qualitative microbiological indicators or a single endpoint without parallel quantitative integrity verification, making it difficult to determine whether microbial control translates into reduced corrosion risk. Second, there is limited formal establishment of a threshold effective concentration (TEC) defined as the minimum concentration that simultaneously achieves microbial suppression (BART negative/non-aggressive) and acceptable corrosion performance (low mass loss/pitting rate). These gaps persist

despite longstanding recommendations for field dose-response testing to avoid under or overdosing (ASTM D4412-19 2019; Pereira et al., 2021).

Although several studies have reported the effectiveness of biocides such as THPS, glutaraldehyde, and ADBAC against SRB (Fajt 2023; Pereira et al., 2021), published information regarding the performance of MB-X in produced-water systems from mature onshore assets remains limited. There are also few reports that have combined SRB-BART aggressiveness classifications with quantitative dose-response assessment to identify an operationally relevant minimum effective dose (MED). Therefore, this study aims to evaluate the performance of biocide MB-X in suppressing SRB growth and aggressiveness in treated water using the SRB-BART method. The experiment also focuses on determining the most effective dose required to reduce SRB populations to Not Aggressive/Negative levels as part of souring and MIC mitigation efforts in oil and gas facilities. The novelty of this study is in establishing a site-specific MB-X dose-response relationship using SRB-BART as well as providing practical recommendations for maintenance and shock dosing strategies in mature produced-water facilities. The results are expected to provide the basis for establishing a cost-effective dose, such as shock versus maintenance, that can be integrated with field corrosion monitoring (corrosion coupons) (Lv et al., 2024; Shi et al., 2023).

2. Methodology

2.1 Study design and objectives

This laboratory-based experimental study was carried out using a quantitative and descriptive method. Observations were conducted to evaluate changes in SRB population and aggressiveness following treatment with the MB-X biocide at different concentrations. A total of three doses (50, 100, and 200 ppm) were tested and compared with a blank control. Effectiveness was assessed using the SRB-BART (Biological Activity Reaction Test) method through daily reaction time (days-to-reaction), tube appearance patterns (BB/BT/BB+BT/Cloudy/Negative), estimated population (CFU/mL), and aggressiveness category (Aggressive/Not Aggressive/Negative) according to HACH guidelines. This method was widely applied for rapid screening and semi-quantitative monitoring of SRB in oilfield facilities. The experimental variables employed in this study are categorized as independent, dependent, and controlled variables as follows:

2.1.A Independent variable (X)

MB-X biocide concentration: 0 ppm (Blank/Control), 50 ppm, 100 ppm, 200 ppm.

2.1.B Dependent variables (Y)

SRB response parameters based on the SRB-BART assay, including:

- Tube appearance
- Time-to-reaction (days) → indicator of SRB population
- Estimated SRB population (CFU/mL) based on days-to-reaction

2.1.C Controlled variables

- Sample volume (uniform for all tubes)
- Type of biocide (MB-X)
- Storage and incubation conditions (room temperature, dark)
- Sampling time

2.2 Sample location and matrix

Produced-water samples were collected from the Outlet Water Treatment of the MX Field within an onshore water-treatment system. SRB are commonly present in process-water pipelines and are associated with biofilms that increase souring/H₂S risk and MIC in carbon steel. Sampling procedures adhered to basic aseptic practices to avoid cross-contamination, thereby preventing contact with vial interiors and using sterile containers, as recommended in BART procedures.

- Inclusion criteria: Fresh samples representing the outlet point at the designated operational schedule were selected.
- Transport and holding: Samples were stored at room temperature, protected from direct sunlight, and analyzed within ≤ 24 hours to preserve Sulfate-Reducing Bacteria viability under Biological Activity Reaction Test conditions.

2.3 Reagents and biocide dosing

The reagents, biocide concentrations, preparation procedures, and control conditions used in this study are described as follows:

- Biocide: MB-X
- Test concentrations: 50, 100, and 200 ppm (v/v or mg/L) in the test water matrix.
- Rationale:
Biocides are commonly applied at concentrations ranging from tens to hundreds of ppm for SRB control in process water, while biofilm-associated SRB typically require higher concentrations than planktonic cells. Therefore, determining the MED under context-specific conditions is recommended before field implementation.
- Preparation:
MB-X stock solution was diluted with sample water to obtain target concentrations. Each treatment was prepared according to laboratory availability and operational constraints. The blank control was evaluated in triplicate, while the 50, 100, and 200 ppm were evaluated using single technical replicates. Consequently, this study was intended as a preliminary dose-response assessment, suggesting that future investigations should include at least three independent replicates per treatment to improve statistical power and reliability.
- Control: Blank sample (no biocide) representing baseline SRB population and aggressiveness.

2.4 SRB-BART assay and incubation

The SRB-BART assay (DBI/Hach) was performed following the manufacturer's instructions:

- 2.4.1 Filling: Add ≥ 20 mL of sample to the outer tube, fill the inner tube to the marked line, reinsert the inner tube, avoid shaking, and allow the floating ball to remain undisturbed.
- 2.4.2 Labeling and incubation: Label with sample ID/date and incubate at room temperature away from direct light.
- 2.4.3 Daily observations: Inspect daily for ≥ 10 days. For deeply infested or slow-growing samples, observations may extend to Day-15 per technical advisories.

Criteria:

- 2.4.4 Positive/Aggressive: Formation of black slime (ring around the ball and/or black precipitate at the bottom).
- 2.4.5 Negative/Non-aggressive: Absence of blackening or cloudiness indicative of SRB.
- 2.4.6 Dominant patterns:
 - BB (black at base): Predominantly anaerobic SRB consortia
 - BT (black at top/ball): Aerobic SRB-associated behavior
 - BB + BT/Cloudy: Mixed populations or presence of other anaerobes.

2.5 Semi-quantitative population estimation (CFU/mL)

SRB population estimates were derived by mapping the first day of visible black reaction to the manufacturer's approximate CFU/mL table:

- Day-1: 2.2×10^6
- Day-2: 5.0×10^5
- Day-3: 1.15×10^5

- Day-4: 2.7×10^4
- Day-5: 6.0×10^3
- Day-6: 1.4×10^3
- Day-7: 3.25×10^2
- Day-8: 75
- Day-9: 20
- Day-10: 5
- Day-11: <1 (represented as 0.5 CFU/mL for log transformation)

These values are widely used as field semi-quantitative indicators.

2.6 Statistical analysis

Analyses were conducted using non-parametric methods because of small sample size and skewed, log-order data. All tests were two-tailed with $\alpha = 0.05$.

- Descriptive:
Median and range (min–max) of CFU and $\log_{10}(\text{CFU/mL})$ per dose, percentage of Aggressive results per dose.
- Group comparison:
[Kruskal–Wallis \(1952\)](#) test for detecting median differences across doses (performed when ≥ 2 groups have $n \geq 1$). Dunn’s post-hoc test planned when replication allows.
- Effect size:
Cliff’s delta compared with the Blank to quantify distributional separation (-1 to $+1$; $|\delta| > 0.474$ shows a large effect for small samples).
- Dose–response:
Spearman rank correlation between dose (ppm) and $\log_{10}(\text{CFU/mL})$ to evaluate monotonic trends.
- Aggressive proportion:
Wilson 95% confidence intervals, which provide stable estimates for small n .
- Fold-reduction:
Median Blank/Median dose as a relative efficacy indicator (range reported; bootstrap CI if replicates sufficient).
- Justification:
Non-parametric tests, non-parametric effect sizes, and rank-based trend statistics are standard for small, skewed count or semi-quantitative datasets. SRB-BART outputs ordinal-continuous population estimates appropriate for this analytical framework.

2.7 Quality assurance/quality control (QA/QC)

To maintain the accuracy and integrity of the experimental results, the following quality assurance and quality control (QA/QC) procedures were applied throughout the study.

- Aseptic method during BART preparation, avoid touching interior surfaces and shaking tubes (prevents false positives/negatives).
- Process control: Blank without biocide, technical duplicates per dose when feasible.
- Interference control: $\text{H}_2\text{S} > 20$ ppm may affect readings, the manufacturer advises simple degassing when high levels are suspected.
- Observation consistency: Daily readings at consistent times, optional photographic documentation for traceability.
- Safety: Biocide and reactive residues handled according to SDS; reaction residues deactivated/sterilized before disposal.

2.8 Ethical, safety, and environmental considerations

Biocide handling followed product SDS and internal HSE requirements. Common oilfield oxidizing and

non-oxidizing biocides, such as ADBAC, THPS, glutaraldehyde, and ClO_2 , require specific safety precautions. Therefore, considerations of effectiveness, risk, and cost were incorporated in dosing decisions.

2.9 Software and reproducibility

Laboratory analyses were conducted at the Production Facility Metering Laboratory operated by PT Pertamina Hulu Rokan, which is routinely utilized for water quality monitoring and microbiological evaluation of oilfield process fluids. All testing procedures were performed in accordance with documented laboratory work instructions and the manufacturer's guidelines for the Sulfate-Reducing Bacteria Biological Activity Reaction Test (SRB-BART). To ensure the reproducibility of test results, samples were analyzed within 24 hours of collection, incubation conditions were maintained under standardized settings, and observations were performed daily at consistent time intervals.

3. Result and discussion

3.1 SRB activity in the untreated sample (blank control)

All three blank samples showed a reaction on Day 3, corresponding to a population of 1.15×10^5 CFU/mL, with a BB tube pattern and an Aggressive interpretation. This result showed a stable and anaerobe-dominant SRB consortium in the produced/treated water, indicating a high sulfidogenic potential typical of mature onshore water systems. The blank dataset was highly consistent across replicates, showing strong baseline SRB activity and validating the suitability of the sample matrix for evaluating biocide performance.

3.2 Effect of MB-X at 50 ppm

The 50-ppm treatment showed the SRB reaction to Day 4, reducing the estimated population to 2.7×10^4 CFU/mL (approximately a 4.3-fold decrease relative to the blank). Despite the reduction, the tube appearance remained BB, and the sample continued to be classified as Aggressive. This suggested that MB-X at 50 ppm provided some inhibitory effect but was insufficient to overcome biofilm-associated SRB resilience. Biofilm protection prevented full penetration of the biocide, a phenomenon widely reported for oilfield SRB communities.

3.3 Effect of MB-X at 100 ppm

At 100 ppm, the microorganism activity was significantly suppressed, with the reaction occurring only on Day 10 (equivalent to 5 CFU/mL). This represents a significant 23,000-fold reduction relative to the blank. Subsequently, the sample transitioned to a Not Aggressive classification, indicating that MB-X at 100 ppm successfully reduced SRB bioburden to levels associated with minimal sulfidogenic and MIC risk. This dose represents a strong candidate for the MED under laboratory conditions.

3.4 Effect of MB-X at 200 ppm

The 200-ppm treatment led to no detectable SRB activity through Day 11, which corresponded to <1 CFU/mL (treated as 0.5 CFU/mL during log analysis). The tube appearance was Negative, suggesting apparent eradication of the microorganisms within the detection limits of the BART method. This performance signifies that 200 ppm is sufficient as a shock dose, typically used in field operations for system cleanup or during outbreaks. The results are consistent with recent studies, indicating that biofilm-associated SRB often require elevated biocide concentrations for complete suppression. Okpala et al. (2026) reported that mature SRB biofilms showed significant tolerance to chemical treatment due to protection by extracellular polymeric substances (EPS). Similarly, Xu et al. (2024) observed that effective control of SRB-related corrosion frequently required concentrations capable of penetrating biofilm structures and disrupting microbial metabolism. The transition from Aggressive (50 ppm) to Not Aggressive (100 ppm) and Negative (200 ppm) observed in this study well with these reported biofilm-control mechanisms.

3.5 Dose response relationship

The dose-response curve produced from $\log_{10}(\text{CFU/mL})$ values shows a strong monotonic decline in SRB population as MB-X concentration increases, progressing from 5.06 (blank) $\rightarrow$ 4.43 (50 ppm) $\rightarrow$ 0.70 (100 ppm) $\rightarrow$ -0.30 (200 ppm). This visually confirms the quantitative pattern observed in the BART results.

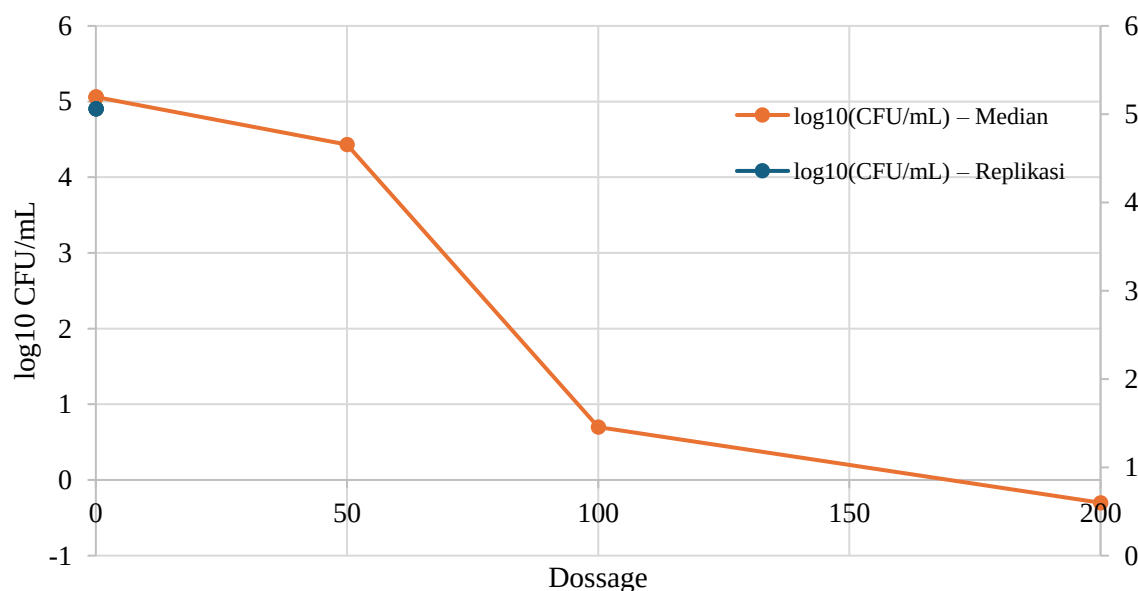


Figure 1.

SRB dose response curve (log 10 CFU/mL).

shows a clear dose-dependent decline in sulfate-reducing bacteria population with increasing MB-X concentration. The most substantial reduction occurs between 50 and 100 ppm, indicating that effective microbial suppression begins within this concentration range

Table 1.
SRB-BART Test Observation Data.

No	Dose-Response Chemical (ppm)	SRB-BART			
		Day in incubation	Tube Appearance	Population (CFU/ml)	Interpretation
1.	Blanko (0)	3	BB	115,000	Aggressive
2.	Blanko (0)	3	BB	115,000	Aggressive
3.	Blanko (0)	3	BB	115,000	Aggressive
4.	Biocide (50)	4	BB	27000	Aggressive
5.	Biocide (100)	10	BB	5.0	Not Aggressive
6.	Biocide (200)	11	Negative	<1 (used 0.5 for log analysis)	Negative

The relationship was further supported by statistical testing:

- Spearman rank correlation showed a perfect monotonic association ($\rho = -1.00$, $p = 0.000$), consistent with a dose-dependent decline.
- Cliff's delta comparisons showed complete stochastic dominance ($\delta = -1.00$ for 50, 100, and 200 ppm vs blank), indicating that all treated values were lower than blank values.
- Kruskal–Wallis results were not statistically significant ($H = 5.00$, $p = 0.172$) due to limited replication, which reduced statistical power despite strong practical differences.
- The apparent inconsistency between the large biological effect observed and the non-significant Kruskal–Wallis result is mainly attributable to the limited and unbalanced replication structure (blank $n = 3$; treatments $n = 1$). Although the hypothesis-testing procedures depend significantly on sample size, effect-size metrics provide a complementary evaluation of practical significance. In this study, both Spearman's rank correlation ($\rho = -1.00$) and Cliff's delta ($\delta = -1.00$) show a strong and consistent dose-dependent response. This suggests that MB-X substantially reduces SRB populations despite the limited statistical power available for formal significance testing.

3.6 Changes in SRB aggressiveness and population structure

The classification trend from Aggressive (0-50 ppm) → Not Aggressive (100 ppm) → Negative (200 ppm) supports the quantitative results. The corresponding Wilson confidence intervals for the proportion of Aggressive outcomes show the small sample size but correlate with the directional shift in SRB behavior. The persistence of BB tube patterns at 50 and 100 ppm shows the underlying SRB consortia remain anaerobic in nature. However, the significant decrease in CFU at 100 ppm suggests that the community structure becomes functionally suppressed despite retained pattern characteristics.

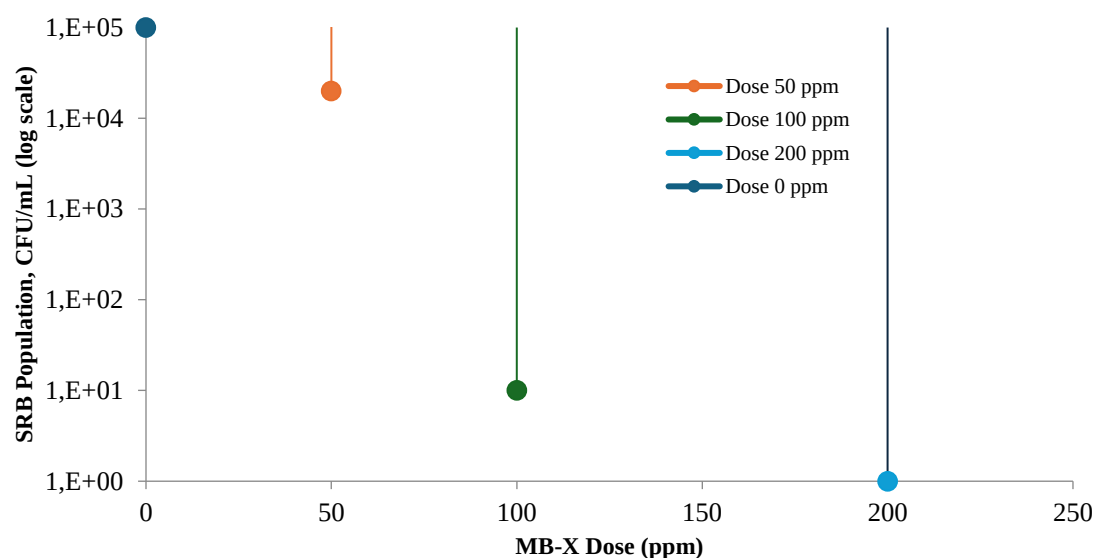


Figure 2.
Median SRB population per dose.
The indicators confirm a robust and consistent dose–response effect,
although the sample size constraints limit inferential statistics.

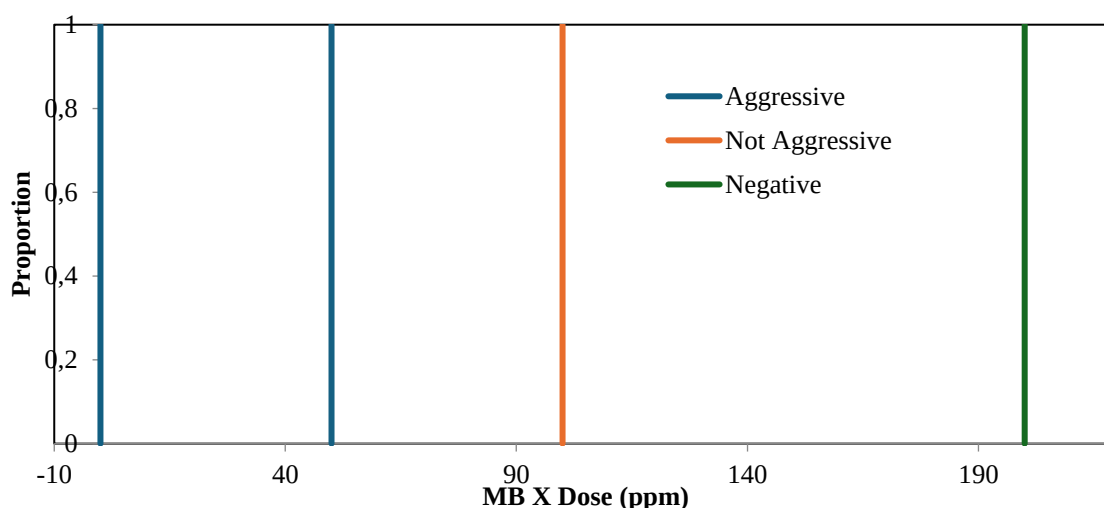


Figure 3.

Aggressiveness categories by dose. A progressive shift from Aggressive to Not Aggressive and finally Negative classification is observed as MB-X concentration increases, demonstrating effective suppression of sulfate-reducing bacterial activity."

3.7 Operational implications for souring and MIC control

Based on the SRB population and aggressiveness indicators:

- 50 ppm is insufficient for operational control and would not reduce souring or MIC risk.
- 100 ppm is effective in shifting SRB behavior to Not Aggressive, which is suitable for maintenance dosing regimens.
- 200 ppm achieves BART-negative conditions, supporting its role as a shock treatment for high-risk or infested systems.

These results are consistent with industry best practices that recommend site-specific MED determination and validation through corrosion monitoring tools such as weight-loss coupons. From a practical field perspective, the results provide a preliminary basis for optimizing biocide treatment programs in produced-water systems. Based on the observed SRB-BART responses, MB-X at 100 ppm can serve as a maintenance dose to sustain SRB populations at Not Aggressive levels during routine operation, while 200 ppm shows potential for shock treatment at elevated microbial activity or confirmed SRB outbreaks. This method is consistent with current recommendations emphasizing site-specific biocide management and performance verification through integrated microbiological and corrosion monitoring programs (Shi et al., 2023; LV et al., 2024). In practice, routine SRB-BART monitoring should be used together with corrosion coupon evaluation to verify that microbial suppression is accompanied by a measurable reduction in MIC risk.

3.8 Limitations and recommendations for future study

Although the experimental outcomes show a clear dose-dependent response of MB-X, the results should be interpreted considering several study limitations. First, treatment groups (50, 100, and 200 ppm) are represented by single technical replicates, which limit statistical power and can underestimate experimental variability. Second, SRB-BART provides semi-quantitative population estimates rather than direct microbial enumeration. Third, this study focuses exclusively on microbiological response and does not include direct corrosion measurements such as coupon weight loss or pitting depth evaluation. Consequently, the observed reduction in SRB activity strongly suggests reduced MIC risk, but direct confirmation through corrosion monitoring remains necessary.

Table 2.
Descriptive statistics and population SRB.

Treatment (ppm)	n	Median CFU/mL	Median log ₁₀ (CFU)	% Aggressive	Fold-reduction vs Blanko (X)	log ₁₀ -reduction (log)
Blanko (0)	3	115000	5.061	100%	1	0
50 ppm	1	27000	4.431	100%	4.259	0.629
100 ppm	1	5	0.699	0% (Not Aggressive)	23000	4.362
200 ppm	1	0.5	-0.301	0% (Negative)	230000	5.362

The SRB-BART data shows a clear and substantial dose–response effect of MB-X against SRB in the produced-/water matrix. Relative to the blank control (median 1.15×10^5 CFU/mL; 100% Aggressive), MB-X achieved significant reductions in microorganism counts and aggressiveness with increasing dose. Specifically, 50 ppm reduced the median to 2.7×10^4 CFU/mL (still 100% Aggressive), 100 ppm to 5 CFU/mL (0% Aggressive; Not Aggressive), and 200 ppm to <1 CFU/mL (0% Aggressive; Negative, treated as 0.5 CFU/mL solely for log-transformation). These medians are consistent with the BART reaction-day mapping used throughout the study and are summarized in Table 2. Quantitatively, the fold-reductions vs. blank were $4.26 \times$ (50 ppm), $2.30 \times 10^4 \times$ (100 ppm), and $2.30 \times 10^5 \times$ (200 ppm), equivalent to log₁₀ reductions of ~0.629, 4.362, and 5.362, respectively. These values show that 50 ppm is sub-effective for changing aggressiveness status. Meanwhile, 100 ppm achieves near-eradication on a logarithmic scale (≥ 4 -log reduction), and 200 ppm reaches BART-negative conditions within the test period.

The dose–response curve of log₁₀(CFU/mL) vs. ppm further supports the observed pattern, with population declining from approximately 5.06 (blank) $\rightarrow$ 4.43 (50 ppm) $\rightarrow$ 0.70 (100 ppm) $\rightarrow$ -0.30 (200 ppm; 0.5 CFU/mL surrogate). This reduction shows monotonic decrease across doses. Spearman's $\rho = -1.00$ ($p \approx 0.000$) confirms a perfect negative rank correlation, while Cliff's $\delta = -1.00$ for 50/100/200 ppm vs. blank shows complete stochastic dominance, with all treated values lower than the blank observations. Although the Kruskal-Wallis test did not reach significance ($H=5.00$; $p=0.172$) due to limited and unbalanced replication (blank $n=3$; each dose $n=1$), the effect magnitude and consistency across metrics were unambiguous. The proportion of Aggressive outcomes also transitions from 100% (blank, 50 ppm) to 0% (100 and 200 ppm). Although the Wilson 95% confidence intervals are necessarily wide because of the small sample size, the categorical results remain directionally consistent with the quantitative response.

From an operational standpoint, the results indicate that MB-X ≥ 100 ppm is sufficient to reduce SRB populations to Not Aggressive levels (≈ 5 CFU/mL) under the tested conditions, supporting application as a maintenance dose. Meanwhile, 200 ppm is suitable as a shock dose for rapid suppression to BART-negative status. The surrogate handling of <1 CFU/mL as 0.5 CFU/mL for log analysis was prespecified and methodologically appropriate for semi-quantitative BART outputs. Therefore, future studies should increase replication to a minimum of three technical or biological replicates per dose to enhance inferential power and enable post-hoc comparisons, including Dunn's test. Microbiology control should also be evaluated alongside in-situ corrosion metrics such as mass loss and pitting rate on coupons to formalize a TEC that simultaneously satisfies microbial and corrosion endpoints.

4. Conclusion

In conclusion, this study successfully evaluates the efficacy of MB-X at multiple doses (50-200 ppm) in suppressing sulfate-reducing bacteria (SRB) activity and population using the Sulfate-Reducing Bacteria Biological Activity Reaction Test. The untreated produced-water sample exhibits high SRB activity and is consistently classified as Aggressive, with a median population of 1.15×10^5 CFU/mL ($\log_{10} = 5.061$), confirming the presence of a strong anaerobic SRB consortium within the tested matrix. MB-X demonstrates a clear dose-

dependent inhibitory effect on SRB, as evidenced by the monotonic decrease in median $\log_{10}(\text{CFU/mL})$ values and the corresponding reduction in aggressiveness from 100% in the blank and 50 ppm treatments to 0% at concentrations of 100 ppm and above. The dose-response relationship further confirms the effectiveness of MB-X in suppressing SRB activity under the laboratory conditions evaluated in this study.

The effectiveness of MB-X varies considerably with dosage. The 50-ppm treatment produces only a modest reduction of 4.26-fold (0.629 log) and fails to change the aggressiveness classification, indicating that this concentration is sub-effective for operational SRB control. In contrast, the 100-ppm treatment achieves a substantial 4.36-log reduction (approximately 23,000-fold), lowering the SRB population to 5 CFU/mL and shifting the interpretation from Aggressive to Not Aggressive. These results indicate that 100 ppm is the most suitable candidate for the minimum effective dose (MED) under the tested laboratory conditions. The 200-ppm treatment provides the greatest level of microbial suppression, producing a BART-Negative response (<1 CFU/mL; surrogate value of 0.5 CFU/mL) and a 5.36-log reduction (approximately 230,000-fold) relative to the untreated condition. This finding suggests that 200 ppm is suitable as an effective shock-treatment dose for system cleanup and high-risk microbial conditions.

Although inferential statistical analysis is limited by the small sample size, the calculated effect-size metric (Cliff's $\delta = -1.00$) and Spearman correlation coefficient ($\rho = -1.00$) provide strong and consistent evidence of MB-X biocidal efficacy across the evaluated dosage range. Based on these findings, a combined treatment strategy consisting of 100 ppm as a cost-effective maintenance dose and 200 ppm as a shock-treatment dose is recommended for field application. Nevertheless, further validation is required to correlate microbial control performance with corrosion-risk reduction outcomes. Future studies should incorporate additional biological and technical replicates together with direct corrosion-performance measurements, including coupon weight-loss and pitting-rate evaluations, to improve statistical robustness and establish a field-validated treatment effective concentration (TEC) for MB-X application in produced-water systems.

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6. Declarations

6.1 Author contributions

Erik Indra Putra: Conceptual, methodology, investigation, laboratory analysis, data curation, original draft preparation, and manuscript revision; Maulana Hardi: Supervision, Validation, technical review, and critical revision of the manuscript; Oktaviani Kusuma Wardani: Writing-review and editing, and manuscript improvement. The authors declare that this manuscript is an original work and has not been published previously, nor is it currently under consideration for publication elsewhere. All data presented are obtained through laboratory testing and operational observations conducted within the scope of this study. The authors have approved the final version of the manuscript and consent to its submission for publication.

6.2 Funding statement

This study received no external funding and was conducted as part of routine technical evaluation, laboratory activities, and key performance indicators fulfillment within the laboratory. All resources, facilities, and operational support required for this study were provided internally by the company.

6.3 Competing interest

The author declares that there are no competing interests, whether financial, professional, or personal, regarding the results, interpretation, and reporting of this study.

6.4 The Use of AI or AI-assisted technologies

The authors used Microsoft Copilot solely for language editing, grammar correction, and improving the readability of the manuscript. Microsoft Copilot was not used for data collection, data analysis, interpretation of results, or generation of scientific conclusions. All content was reviewed and approved by the authors, who take full responsibility for the final manuscript.

7. Glossary of terms and symbols

Terms & symbols	Definition	Unit
SRB	Sulfate-Reducing Bacteria; anaerobic microorganisms that use sulfate as a terminal electron acceptor and produce hydrogen sulfide (H ₂ S)	–
Sulfidogenesis	Biological production of sulfide (H ₂ S) by SRB during anaerobic respiration	–
MIC	Microbiologically Influenced Corrosion; deterioration of metals accelerated or mediated by microbial activity	–
FeS	Ferrous sulfide: black corrosion product commonly formed in SRB-impacted systems	–
Biofilm	Structured microbial community embedded in an extracellular polymeric substance (EPS) matrix attached to surfaces	–
EPS	Extracellular Polymeric Substance protecting microorganisms within biofilms	–
Produced/Treated Water	Water originating from oil and gas production and/or water-treatment systems	–
Waterflooding	Secondary recovery method involving injection of water to maintain reservoir pressure and hydrocarbon displacement	–
SRB-BART	Biological Activity Reaction Test is used for rapid detection and semi-quantitative monitoring of SRB	–
Days-to-Reaction	On the first day, visible blackening appears in BART and is used to estimate SRB population	day
BB	Black-at-Base pattern showing anaerobic SRB consortia	–
BT	Black-at-Top (Ball) pattern showing activity near the air-liquid interface	–
BB+BT	Mixed blackening pattern showing mixed microbial populations	–
Cloudy	Turbid appearance showing mixed microorganisms or other anaerobes	–
Negative	No blackening or clouding observed during incubation period.	–
Aggressiveness Category	Qualitative BART classification (Aggressive, Not Aggressive, Negative)	–
CFU/mL	Colony-Forming Units per milliliter; semi-quantitative microbial population estimate	CFU/mL

<1 CFU/mL Handling	Values below detection represented as 0.5 CFU/mL for log transformation	CFU/mL
Biocide	Chemical agent used to inactivate or control microbial populations	ppm or mg/L
Shock Dose	High-concentration, short-duration biocide treatment for rapid microbial reduction	ppm
Maintenance Dose	Lower concentration biocide treatment for routine microbial control	ppm
MED	Minimum Effective Dose; lowest concentration achieving desired microbiological endpoint	ppm
TEC	Threshold Effective Concentration; minimum concentration satisfying microbiological and corrosion criteria	ppm
Fold Reduction	Ratio of baseline microbial population to treated population	×
Log ₁₀ Reduction	Base-10 logarithmic decrease in microbial population	log
Dose-Response Curve	Relationship between biocide concentration and microbial response	–
Kruskal-Wallis Test	Non-parametric statistical test used to compare medians across multiple groups	–
Dunn's Test	Non-parametric post-hoc pairwise comparison test following Kruskal-Wallis	–
Spearman's Rank Correlation (ρ)	Rank-based correlation coefficient measuring monotonic relationships	–
Cliff's Delta (δ)	Non-parametric effect-size statistics showing stochastic dominance between groups	–
Wilson Confidence Interval	Confidence interval method for binomial proportions, specifically suitable for small samples	%
Median ($\tilde{x}$)	Robust measure of central tendency for skewed data	Same as measured variable
Range (Min-Max)	Measure of dispersion showing minimum and maximum observed values	Same as measured variable
13Cr Steel	Martensitic stainless steel commonly evaluated in MIC and souring studies.	–
Corrosion Coupon	Metal specimen used to quantify corrosion rates, metal loss, and pitting tendencies	mm/y, mpy
HSE	Health, Safety, and Environment framework for managing occupational and environmental risks	–
SDS	Safety Data Sheet containing chemical hazards and handling information	–
QA/QC	Quality Assurance and Quality Control procedures ensuring data integrity and reproducibility	–
OPEX	Operating Expenditure associated with operational and maintenance activities	Currency/year
MB-X	Proprietary biocide evaluated in this study	ppm or mg/L
ADBAC	Alkyl Dimethyl Benzyl Ammonium Chloride; quaternary ammonium non-oxidizing biocide	ppm or mg/L
THPS	Tetrakis(hydroxymethyl)phosphonium sulfate; non-oxidizing oilfield biocide	ppm or mg/L
Glutaraldehyde	Broad-spectrum non-oxidizing biocide	ppm or mg/L
ClO ₂	Chlorine dioxide: oxidizing biocide used for microbial and H ₂ S control	ppm or mg/L
H ₂ S	Hydrogen sulfide generated through SRB metabolism	mg/L or ppm
ppm	Parts per million concentration unit; approximately equivalent to mg/L in aqueous systems	

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