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Optimisation Review of a Novel Chemistry for Enhanced Microbiological Souring Remediation and Mitigation under Simulated and Realistic Field Trial Conditions

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Introduction and Industry Innovation demands

Reservoir souring caused by biogenic hydrogen sulphide (H_2S) generation remains a critical operational and integrity challenge for the global oil and gas industry. Biogenic souring is primarily driven by the activity of sulphate-reducing microorganisms (SRM), which utilise sulphate present in injected or formation waters as an electron acceptor, producing H_2S as a metabolic by-product. The consequences of biogenically derived souring include increased corrosion risk, health and safety hazards, reduced hydrocarbon value, and significant operational expenditure associated with mitigation and remediation.

Historically, souring control strategies have focused on preventative approaches, most notably the application of conventional biocides, nitrate injection, and sulphate removal units (SRUs). While nitrate treatment can suppress sulphide generation through competitive microbial pathways and SRUs reduce sulphate availability, both strategies are often capital-intensive and operationally complex.

Conventional, generic biocides, although widely deployed, typically offer limited long-term efficacy under reservoir conditions, frequently exhibiting strong preventative capacity but limited remediation performance once souring is established. As a result, many field applications rely on continuous or high-frequency dosing regimes, leading to elevated OPEX with variable and often short-lived success.

Despite decades of industry effort, the development of a robust, cost-effective, and sustainable solution for long-term microbiological souring control has achieved limited success. Ongoing research has increasingly highlighted the need for alternative strategies that move beyond short-term microbial suppression toward durable system control. In particular, there is growing interest in novel biocidal and non-biocidal chemistries capable of functioning synergistically with reservoir microbiology, providing both enhanced remediation of established souring and long-term preservative control.

This paper presents a laboratory-based screening evaluation of a novel biocide chemistry under reservoir-simulated conditions, alongside a comparative assessment against established, generic industry biocides. The objective is to assess the potential operational value, system-

level control, and long-term preservative capability of this chemistry for effective biogenic souring mitigation. Given that reservoir souring represents a persistent pinch point across global oil and gas operations, the development of robust and effective biogenic souring control solutions remains essential for sustainable field management.

Bioreactor Design and Execution

High-pressure, flowing bioreactors as shown in **Figure 1** are critical tools for accurately evaluating microbial souring as they replicate the key physical and chemical conditions present within the downhole reservoir environment (Dutta et al., 2020; Nixon et al., 2017; Streets et al., 2025).

Pressure, temperature and fluid flow strongly influence microbial metabolism, community structure and reaction kinetics, particularly for sulphate-reducing microorganisms responsible for subsurface hydrogen sulphide generation (Eden et al., 1993). Laboratory tests conducted under ambient and static conditions often fail to capture these effects, leading to an incomplete or misleading understanding of souring risk (Streets & Eden, 2024). By simulating in situ pressures, elevated temperatures and dynamic flow regimes, high-pressure bioreactors allow microbial behaviour to be assessed under conditions that more closely reflect those controlling souring in the field.

These systems are equally important when evaluating the performance and limitations of chemical remediation strategies (Dutta et al., 2020; Jones et al., 2011; Streets et al., 2025). The efficacy, transport and persistence of nitrates, biocides and other souring control chemistries are highly sensitive to pressure-driven phase behaviour, temperature-dependent reaction rates and flow-controlled residence times.

Flowing bioreactors enable realistic assessment of chemical–microbial interactions, including inhibition efficiency, rebound potential and shifts in microbial community composition following treatment. This improves confidence in laboratory-to-field translation, reduces uncertainty in chemical selection and dosing strategies and ultimately supports more robust, cost-effective and lower-risk souring management programmes.

Figure 1: Bioreactor Set-up for the executed study basis (Rawwater Engineering Company Limited, UK).

Preservative vs. Preventative Biocide Chemistry

Reservoir souring, mainly caused by sulphate-reducing microorganisms (SRM) and other anaerobic microorganisms, is a persistent challenge in water injection, EOR, and long-term reservoir operations. Once established, microbial activity generates hydrogen sulphide, leading to corrosion, loss of injectivity, safety risks, and reduced asset integrity. Effective souring mitigation therefore requires not only an initial reduction of microbial load, but, more importantly, sustained metabolic recovery and suppressive control of bacterial regrowth deep in the reservoir.

Preservative-type biocides are central to this approach. Unlike preventative "quick killing" biocides that act rapidly but are depleted within hours or days, long-term preservatives are designed to remain stable and active over extended periods. This enables continuous suppression of microbial activity during water storage, injection, reservoir residence, and well shut-in phases, when biogenic souring risk is highest.

3,3′-Methylenbis(5-methyl-1,3-oxazolidin) (MBO, CAS 66204-44-2), manufactured and marketed by Vink Chemicals as grotan® OX, represents a proven preservative chemistry for reservoir souring mitigation. The broad antimicrobial spectrum of the oxazolidine based active component effectively targets sulphate-reducing bacteria (SRB), acid-producing bacteria (APB), and general heterotrophic bacteria (GHB), supporting long-term reservoir integrity. grotan® OX is highly compatible with challenging oilfield conditions, including high salinity brines, elevated temperatures (up to 160 °C), and mixed oil/water systems. Its non-ionic nature minimises adsorption onto formation rock and equipment surfaces, ensuring that active biocide remains available in the bulk phase and can penetrate deeper into the reservoir.

A key advantage of preservative biocide chemistry is its multifunctional, dual mode of action. In addition to long-term biocidal performance, grotan® OX interacts with hydrogen sulphide present in partially soured systems. Rather than being rapidly deactivated, the active material reacts with H_2S to form a stable organic sulphur compound with significantly reduced toxicity and corrosivity. Importantly, this reaction does not represent simple loss of activity through scavenging; the reaction products can retain meaningful antimicrobial efficacy, enabling a '2-in-1' treatment concept that combines microbial control with H_2S risk mitigation.

With > 99% active content, favourable handling properties, biodegradability, and global regulatory approvals (FIFRA, BPR, CEFAS, NEMS), grotan® OX delivers a robust, valued and effective solution for long-term reservoir souring control, where controlled preservation, not solely a single biocidal treatment, determines success.

Novel Chemistry for Biogenic Souring Control

grotan® OX is a condensation reaction product, and its mode of action is governed by controlled hydrolysis in the fluid matrix. The biocidal active species is not present at high concentration initially but is generated progressively as hydrolysis begins. The rate of hydrolysis, and therefore biocide availability, depends on system parameters such as concentration, pH, temperature, and residence time.

Hydrolysis is a reversible chemical equilibrium in which the active converts to intermediate species and ultimately to the corresponding educts. As the active species is consumed through microbial interaction or side reactions in the system, it is continuously re-formed from the

original active molecule. This dynamic equilibrium ensures that only low concentrations of active biocide are present at any given time, minimizing rapid depletion while maintaining long term antimicrobial efficacy.

This controlled, slow-release mechanism underpins the long-term preservative behaviour of the chemistry, enabling sustained microbial control during water storage, injection, reservoir exposure, and well shut-in. The result is prolonged system persistence, reduced need for frequent re-dosing, and reliable performance under the extended protection times required for reservoir souring mitigation.

Study Execution and Key Parameters

In total, ten water-saturated, high-pressure bioreactors were constructed of 316L stainless steel and the five sets of duplicates operated in parallel at the Rawwater UK laboratories to evaluate microbial processes under representative downhole conditions for a total of 17 weeks. All bioreactors were maintained under pressurised, anoxic conditions at 1,000 psi g (69 bar g) and 30°C, selected to simulate near wellbore environments commonly encountered in oilfield seawater injection and reservoir systems.

Bioreactors were inoculated through the packing of a pre-established, hydrocarbon-containing sand matrix, populated with a mature, diverse, sessile (biofilm) oilfield microbial community. The use of a developed biofilm within a water-saturated porous medium allowed realistic simulation of microbially active reservoir and near wellbore zones, where sessile communities dominate sulphate reduction and hydrogen sulphide generation (R. De Paula et al., 2023; R. M. De Paula et al., 2024; Streets et al., 2025). Parallel operation of the ten bioreactors provided experimental robustness through the use of replicates, improving reproducibility and accounting for the inherent variability of complex microbial systems, which can exhibit differing responses under otherwise identical, tightly controlled and field-relevant conditions.

During each weekly batch injection cycle, a baseline injection fluid comprising anoxic synthetic seawater supplemented with 120 mg/l mixed volatile fatty acids (VFAs) was injected to provide a representative carbon source typical of oil-bearing reservoir environments. The VFA mixture consisted of acetate, propionate and butyrate at a ratio of 100:10:10, supporting sustained microbial activity within the bioreactors. Predicted sulphide production was approximately 71 mg/l and all stoichiometric calculations, based on molecular weights of the relevant compounds, were performed to estimate sulphate reduction and sulphide generation. Injection cycles were conducted at a controlled flow rate of 5.0 ml/min to maintain consistent hydraulic and nutrient delivery conditions across all systems.

In total, four chemical treatments were evaluated and compared against a biological control set, as outlined below:

A. **Glutaraldehyde (50% Active)**
B. **THPS (50% active)**
C. **Vink Chemicals In-house Candidate (*confidential*) (> 95% active)**
D. **Vink Chemicals grotan® OX (99% Product active / 47% Released Active)**
   Dosages of 750 ppm/v utilised based on active material loading equivalence.

During the first three injection cycles, one third of the measured swept volume in each bioreactor was replaced using the baseline injection water (60 ml injection cycle). In week 4,

the full swept volume of each bioreactor column was displaced (180 ml injection cycle) to re-establish consistent baseline conditions prior to the biocide dosing phase in week 5.

Biocides were applied at concentrations representative of typical field-use levels, with all treatments dosed across the full swept volume of the designated bioreactors at 750 mg/l product concentration (180 ml injection cycle). Following biocide application, the bioreactors received one-third swept volume injections between weeks 6 and 17 (60 ml injection cycle). This operational approach enabled real-time assessment of biocide efficacy, microbial recovery and long-term treatment persistence under flowing, pressurised conditions.

Key Surveillance Methodologies

A range of analytical techniques was applied throughout the study to both aqueous and core samples to monitor and characterise microbial activity, summarised in **Table 1**.

Bioreactor produced water sulphide concentration was measured weekly using zinc acetate capture, followed by methylene blue colorimetric analysis with a limit of quantification (LoQ) set to 0.5 mg/l. Ion chromatography was used to determine individual VFA concentrations (LoQ set to 0.2 mg/l for each VFA component) of the injection water and of the bioreactor produced waters each week. Aqueous pH measurements were also recorded weekly for both injection fluids and bioreactor produced water samples to monitor temporal changes in system chemistry.

Throughout the study, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) concentrations were quantified using luminescence-based assays (Luminultra Technologies Ltd, Canada). Combined, the measurements were used to calculate the AMP Index (AMPi) and the Adenylate Energy Charge (AEC). These measurements were used to infer microbial metabolic load, overall community activity and physiological stress responses across all bioreactors before and after the biocide application.

Most Probable Number (MPN) enumeration was also used throughout the project to estimate the culturable planktonic GHB and SRB populations from injection water and produced water samples, using phenol red dextrose (PRD) and modified Postgate B (MPB) media respectively.

Sessile microbial communities of the starting pre-established sand matrix inoculum and end point decommissioned core samples (week 17) were characterised using quantitative PCR (qPCR) and 16S rRNA gene sequencing, NGS (Luminultra Technologies Ltd, Canada). The qPCR targets selected were Total Prokaryotes, Sulphate-Reducing Prokaryotes and Sulphur-Oxidising Bacteria.

Table 2: Overview of the surveillance and analytical methodology applied throughout the 17-week study, with shaded cells indicating time points at which specific analyses were performed.

Analysis	Project Week																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sulphide																	
VFA																	
pH																	
AMPi/AEC																	
MPN																	
qPCR																	
NGS																	

Multiple Lines of Evidence Criticality

Any reservoir souring review, biocide selection programme, and associated laboratory study execution leading toward a pilot field trial must be grounded in a rigorous assessment and clear understanding of the underlying control mechanisms.

Effective souring mitigation is not achieved through product screening alone, but through validation of how and why a given treatment influences microbial activity under representative field conditions. This validation must be supported by multiple, independent lines of evidence spanning biochemical, chemical, and microbiological assessments.

Modern reservoir souring studies increasingly combine:

- Produced fluid and water chemistry (sulphate, H_2S levels)
- Injection and flow history data
- Microbial / qPCR / molecular analyses (when available)
- Numerical modelling and uncertainty quantification
- Geochemical, isotopic, and rock-fluid interaction evidence

to distinguish biogenic vs. thermogenic sources, quantify souring stages, and predict future souring trends with higher confidence. This integrated approach provides a comprehensive mechanistic understanding rather than isolated observations.

Critically, laboratory studies should be designed to differentiate between distinct modes of souring control, whether related to direct sulphide production suppression, volatile fatty acid (VFA) limitation, or inhibition of key metabolic pathways within sulphate-reducing and associated microbial communities. The aligned use of biochemical tools such as ATP/ADP/AMP loading, adenylate energy charge (AEC) evaluation, and metabolic flux indicators provide insight into microbial viability, stress response, and energy limitation that conventional culture-based techniques alone cannot resolve. These approaches allow direct assessment of metabolic inhibition rather than relying solely on organism presence or absence.

At the same time, conventional microbiological methods—including selective culturing and MPN techniques—retain value when interpreted within their known limitations. They provide continuity with historical datasets and can offer qualitative confirmation of treatment trends when combined with molecular or biochemical data. However, reliance on any single methodology risks oversimplifying complex microbial ecosystems and may lead to incorrect conclusions regarding treatment efficacy or longevity.

The true technical value lies in integrating these differing methodologies into a coherent, fit-for-purpose selection framework. By combining biochemical indicators of metabolic suppression, chemical measurements of sulphide and VFA dynamics, and microbiological assessments using both culture-based and non-culture-based techniques, operators can develop a robust, defensible basis for biocide selection.

This integrated approach significantly reduces uncertainty when transitioning from laboratory studies to pilot field trials, ensuring that observed laboratory performance reflects genuine control mechanisms rather than artefacts of test design or analytical bias.

Study Phase 1 – Sulphide Generation and VFA Utilisation

The establishment of a primary performance parameter and the definition of a measured outcome against which suitable chemistries can be screened is absolutely fundamental to any souring control evaluation study.

In the present work, all bioreactors provided a suitable and reproducible baseline, together with a well-controlled bioenvironment, in which volatile fatty acid (VFA) utilisation and sulphide generation from a mixed marine microbial consortium could be readily achieved.

This stable baseline represented a critical first stage prior to the execution of any chemical study basis, ensuring that subsequent performance observations could be attributed with confidence to the chemistry properties and action rather than biological instability, nutrient depletion and starvation or reactor artefacts.

The ability to reliably link VFA consumption with sulphide evolution is particularly important, as these parameters are directly coupled to the metabolic activity of sulphate-reducing and associated anaerobic microorganisms responsible for biogenic souring through H_2S liberation. Once established, these two key chemical parameters were tracked on a regular and consistent basis throughout the duration of the study. Their continued monitoring allowed for both short-term response assessment following chemical application and longer-term evaluation of sustained microbial control.

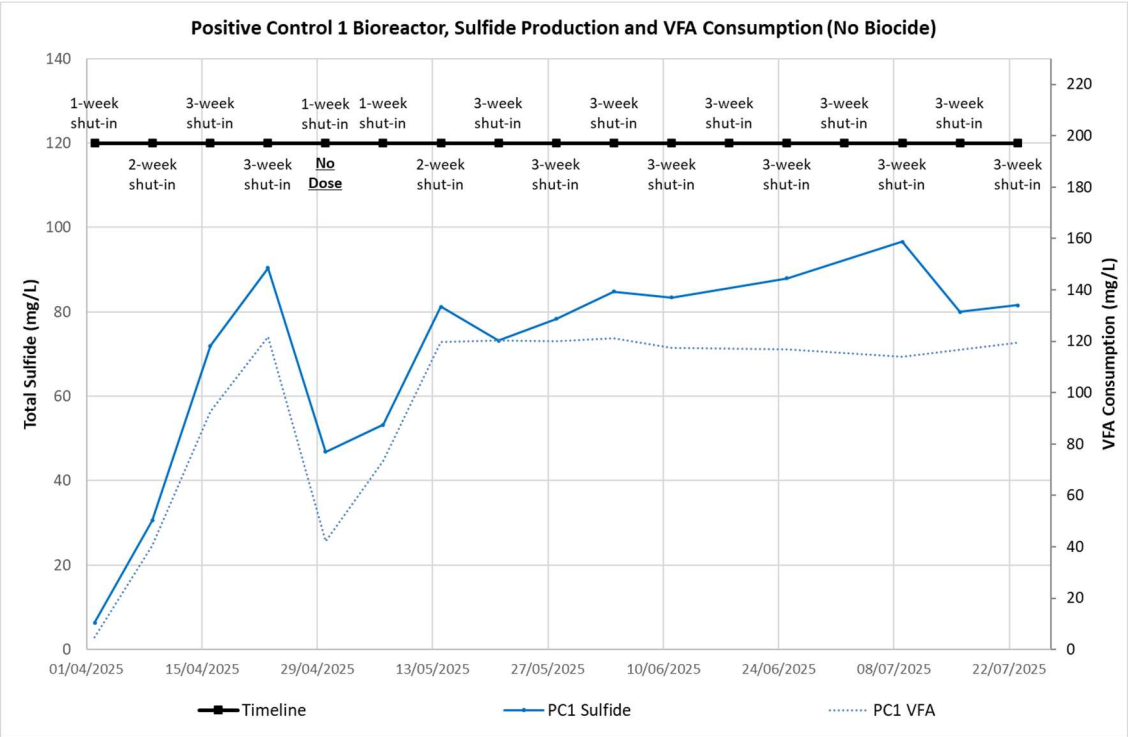
Within this framework, a reduction in evolved sulphide, when matched with a significant reduction in unutilised VFA content, was interpreted as a robust and technically defensible indicator of effective biogenic souring control. This combined response demonstrates not only suppression of sulphide-producing activity, but also inhibition of upstream fermentative processes that supply metabolic substrates to sulphate-reducing populations.

Conversely, scenarios in which sulphide reduction occurred without a corresponding decrease in VFA were treated with caution, as these may indicate temporary metabolic inhibition, sulphide scavenging effects, or pathway redirection rather than true microbial control.

By anchoring chemical screening to these tightly coupled parameters, the study provides a rigorous and mechanistically sound basis for comparing candidate chemistries and assessing their suitability for long-term souring control applications under representative marine conditions.

As demonstrated below in **Figure 2**, the PC1 control bioreactor dataset recorded over the full study period tracks both sulphide generation and volatile fatty acid (VFA) utilisation. The data clearly verify that an actively biogenic souring consortium was present, metabolically active, and thriving under the imposed experimental conditions. Throughout the study duration, no evidence of nutrient limitation is observed, nor was there any retardation in either the rate or cumulative volume of evolved H_2S. Sulphide production and VFA consumption remained well aligned, confirming stable microbial activity and sustained sulphate-reducing bacterial performance. This outcome was critical, as it validated the control system and confirmed that any subsequent changes observed in treated systems can be confidently attributed to the applied chemistry rather than artefacts of biological instability or nutrient depletion.

Figure 2: Bioreactor total sulphide (mg/l) generation and VFA consumption (mg/l) vs. time for the study period (PC1 – Positive Controls (no biocide)).



An equivalent chart format is presented in **Figure 3** for the D1 bioreactor treated with grotan® OX at 750 ppm/v.

Prior to the biocide dosing point, the reactor exhibits a biogenic souring capacity, which aligned with the untreated control, confirming comparable baseline microbial activity. Following biocide application, a clear and sustained control of both sulphide generation and VFA consumption is observed. The concurrent suppression of these two parameters indicated effective inhibition of the active souring consortium rather than a transient or partial metabolic disruption.

These results allow the conclusion that a single biocide dose delivered both immediate souring control and a prolonged preservative effect. The sustained suppression observed post-dosing supported the suitability of the chemistry for long-term biogenic souring control evaluation and provides a robust basis for extrapolating laboratory performance to controlled field deployment scenarios.

Figure 3: Bioreactor total sulphide (mg/l) generation and VFA consumption (mg/l) vs. time for the study period (D1 – grotan® OX 750 ppm/v)).

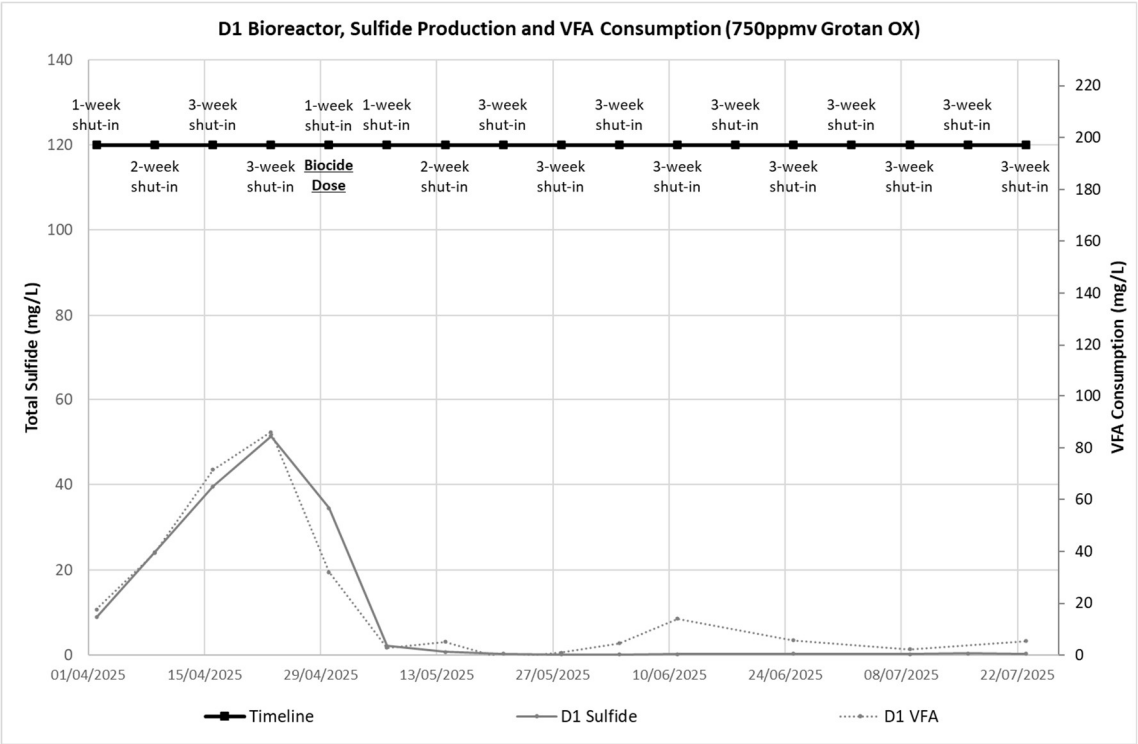


Figure 4 presents the complete bioreactor dataset generated during the study, illustrating total sulphide production (mg/l) as a function of time for all tested conditions. The duplicate positive controls (PC1 and PC2) demonstrate closely aligned sulphide generation profiles, confirming the reproducibility of the system and the stability of the actively souring microbial consortium under untreated conditions.

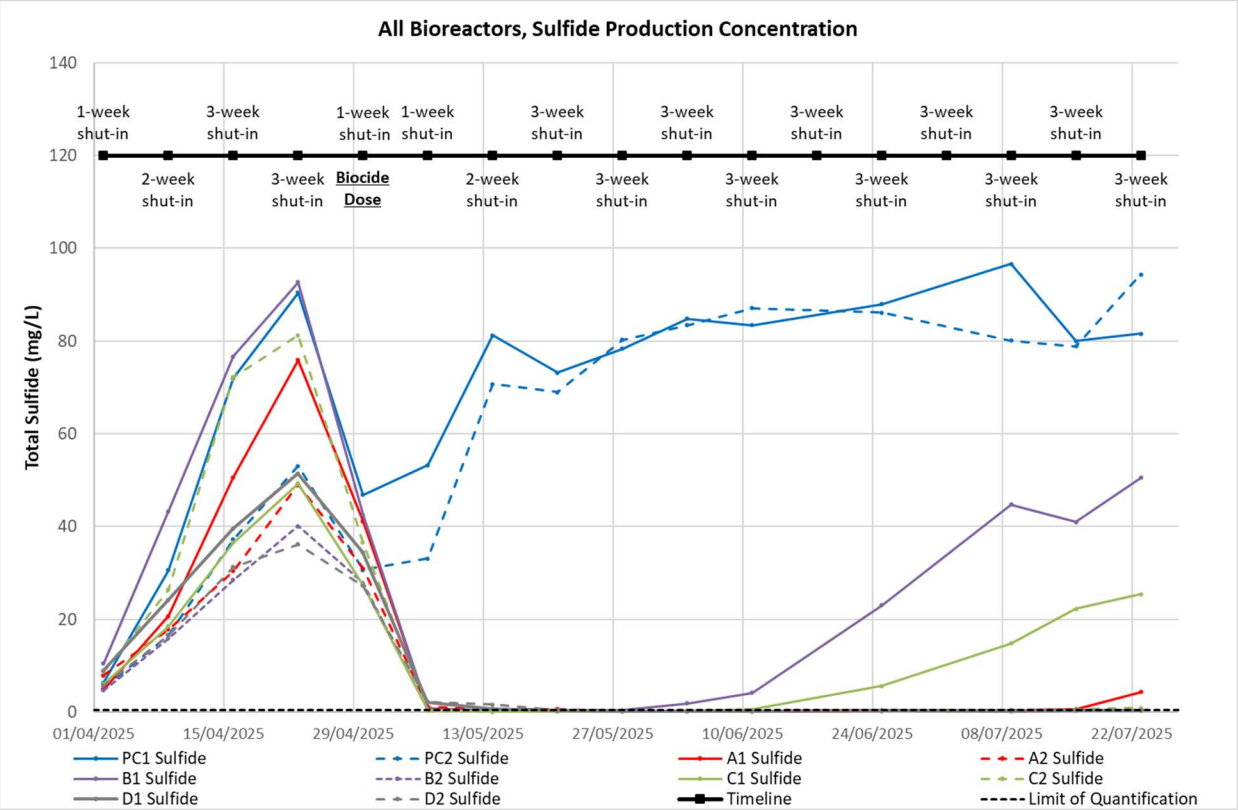
A clear differentiation in biogenic sulphide control is observed across the biocide-treated reactors. In the case of B1 (THPS at 750 ppm/v), sulphide regeneration was detected approximately six weeks after biocide dosing, indicating a relatively short microbial hold period compared with the other chemistries evaluated. The in-house candidate (C1, 750 ppm/v) exhibited sulphide recovery after approximately eight weeks, demonstrating improved but still limited persistence under the applied conditions.

Glutaraldehyde at 750 ppm/v (A1) showed significantly extended souring control, with sulphide suppression maintained until approximately week 15 of the 17-week study. While this represented strong long-term performance, partial recovery was still evident toward the end of the test period and souring control is lost.

In contrast, grotan® OX at 750 ppm/v, evaluated in duplicate bioreactors D1 and D2, was the only chemistry to demonstrate complete and sustained sulphide control throughout the entire extended study duration in both bioreactors. No sulphide recovery or renewed generation was observed at any point, indicating robust microbial inhibition and a persistent preservative effect.

Overall, **Figure 4** clearly differentiates both the effectiveness and longevity of the evaluated chemistries, with grotan® OX delivering optimal and uninterrupted souring control under the defined experimental pressure, temperature and flow conditions.

Figure 4: Bioreactor total sulphide (mg/l) generation vs. time for the study period (PC1/2 – Positive Controls (no biocide), A1/A2 – Glutaraldehyde 750ppm/v, B1/B2 – THPS 750ppm/v, C1/C2 – In House Candidate 750 ppm/v, D1/D2 – grotan® OX 750 ppm/v).



Study Phase 2 – ATP, AEC and Metabolic Stress

As a secondary line of evidence, ATP surveillance was conducted on bioreactor liquid sub-samples on a regular basis to assess overall microbial load equivalence. ATP measurement is not a direct indicator of microbial activity; rather, it is a chemically based assay used to quantify equivalent microbial loading within the system.

To enhance interpretive value, additional measurements of ADP and AMP were performed. These lower-energy adenylate molecules are intrinsically linked to the ATP-driven cellular energy cycle and provide insight into microbial energetic status and stress response.

The combined values of ATP, ADP and AMP data were used to calculate both the AMP Index and the Adenylate Energy Charge (AEC). These calculated parameters are well-established indicators for evaluating microbial stress and viability.

- AMP Index values of <0.1 indicate no measurable microbial stress; values >0.1 to 1.0 indicate minor stress; 1.0–3.0 indicate moderate microbial stress; 3.0–10.0 indicate lethal microbial stress; and values >10.0 indicate sustained lethal stress for a minimum duration of one day.

- AEC values are used to assess microbial viability and metabolic state. An AEC >0.8 is characteristic of actively growing microbial populations; values between 0.5 and 0.8

indicate viable but non-proliferating cells; and values <0.5 are associated with dormancy or cell death.

Together, these assessments allow differentiation between immediate microbial activity control (e.g. biocidal action) versus the potential for longer-term suppression of microbial recovery. This distinction is critical for evaluating treatment consistency under conditions where partial microbial recovery may persist and contribute to renewed biogenic souring risk.

ATP-based metabolic indicators were used to evaluate microbial activity, stress response, and recovery dynamics across untreated and biocide-treated bioreactors over the 17-week study. The untreated control bioreactors (PC1/PC2) exhibited a relatively stable Adenylate Energy Charge (AEC) throughout the entire operational period. This stability is indicative of a metabolically active and healthy microbial population, with no meaningful disruption to energy balance or growth conditions. In parallel, the Adenylate Monophosphate index (AMPi) remained consistently low and stable in the controls, confirming limited to no physiological stress and validating the robustness of the baseline system.

In contrast, as shown in **Figures 5 and 6**, all biocide-treated bioreactors demonstrated a pronounced shift in both AEC and AMPi during week 6, immediately following biocide dosing in week 5. AEC values declined sharply from levels associated with active growth toward ranges consistent with dying or dormant microbial states, demonstrating effective disruption of cellular energy metabolism. This response was mirrored in AMPi data, which showed a marked increase indicative of lethal stress conditions across treated systems.

Among the biocides evaluated, grotan® OX was the only product to deliver sustained lethal stress across both duplicate bioreactors at weeks 6 and 8 (i.e., in samples where bioreactor produced water was associated with the week 5 biocide treatment), highlighting superior consistency and robustness. Importantly, AEC and AMPi were not only used to assess immediate kill performance, but also to track microbial recovery potential. While other treatments showed partial rebound toward active metabolic states over time, grotan® OX maintained AEC data points within dying or dormancy zones through to week 17, demonstrating long-lasting suppression and a strong hold on microbial recovery.

Figure 5: Bioreactor AEC and AMP Index review vs. time for the study period (PC1/2 – Positive Controls (no biocide), A1/A2 – Glutaraldehyde 750ppm/v, B1/B2 – THPS 750ppm/v, C1/C2 – In House Candidate 750 ppm/v, D1/D2 – grotan® OX 750 ppm/v).

Bioreactor PC1/PC2 – Controls (No Biocide)

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	648	186	0	0.89	Actively Growing	0.50	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose (No Biocide)**	341	22	135	0.71	Viable, but not Growing	0.89	Minor Microbial Stress
07/05/2025	6	1 week post-dose	679	223	122	0.77	Viable, but not Growing	1.18	Microbial Stress
21/05/2025	8	3 weeks post-dose	715	417	175	0.71	Viable, but not Growing	0.20	Minor Microbial Stress
04/06/2025	10	5 weeks post-dose	1010	0	1	1.00	Actively Growing	0.60	Minor Microbial Stress
25/06/2025	13	8 weeks post-dose	627	253	202	0.70	Viable, but not Growing	0.54	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	782	516	225	0.68	Viable, but not Growing	0.06	No Microbial Stress

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	1414	1012	310	0.70	Viable, but not Growing	1.22	Microbial Stress
30/04/2025	5	**Immediately pre-dose (No Biocide)**	2145	0	295	0.88	Actively Growing	0.54	Minor Microbial Stress
07/05/2025	6	1 week post-dose	995	272	125	0.81	Actively Growing	1.08	Microbial Stress
21/05/2025	8	3 weeks post-dose	1479	804	337	0.72	Viable, but not Growing	0.18	Minor Microbial Stress
04/06/2025	10	5 weeks post-dose	1959	0	0	1.00	Actively Growing	0.59	Minor Microbial Stress
25/06/2025	13	8 weeks post-dose	1756	308	168	0.86	Actively Growing	0.28	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	1651	1624	654	0.63	Viable, but not Growing	0.15	Minor Microbial Stress

Bioreactor A1/A2 – Glutaraldehyde 750 ppm/v

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	1085	643	73	0.78	Viable, but not Growing	0.94	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	1089	71	422	0.71	Viable, but not Growing	0.88	Minor Microbial Stress
07/05/2025	6	1 week post-dose	6	13	14	0.39	Dying or Dormant	4.79	Lethal Microbial Stress
21/05/2025	8	3 weeks post-dose	1	5	7	0.24	Dying or Dormant	10.52	Lethal Stress for at Least One Day
04/06/2025	10	5 weeks post-dose	9	0	4	0.70	Viable, but not Growing	1.27	Microbial Stress
25/06/2025	13	8 weeks post-dose	5	2	8	0.43	Dying or Dormant	1.82	Microbial Stress
23/07/2025	17	12 weeks post-dose	31	32	18	0.58	Viable, but not Growing	0.30	Minor Microbial Stress

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	3536	2053	110	0.80	Actively Growing	0.88	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	5284	0	0	1.00	Actively Growing	0.29	Minor Microbial Stress
07/05/2025	6	1 week post-dose	6	3	12	0.35	Dying or Dormant	4.67	Lethal Microbial Stress
21/05/2025	8	3 weeks post-dose	1	3	7	0.20	Dying or Dormant	11.39	Lethal Stress for at Least One Day
04/06/2025	10	5 weeks post-dose	2	2	5	0.30	Dying or Dormant	5.39	Lethal Microbial Stress
25/06/2025	13	8 weeks post-dose	11	4	8	0.55	Viable, but not Growing	1.07	Microbial Stress
23/07/2025	17	12 weeks post-dose	14	11	10	0.57	Viable, but not Growing	0.38	Minor Microbial Stress

Bioreactor B1/B2 – THPS 750 ppm/v

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	376	109	0	0.89	Actively Growing	0.61	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	516	0	100	0.84	Actively Growing	0.62	Minor Microbial Stress
07/05/2025	6	1 week post-dose	2	71	11	0.45	Dying or Dormant	8.98	Lethal Microbial Stress
21/05/2025	8	3 weeks post-dose	1	4	8	0.25	Dying or Dormant	7.22	Lethal Microbial Stress
04/06/2025	10	5 weeks post-dose	48	1	4	0.92	Actively Growing	0.71	Minor Microbial Stress
25/06/2025	13	8 weeks post-dose	44	14	13	0.72	Viable, but not Growing	0.51	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	40	44	23	0.58	Viable, but not Growing	0.31	Minor Microbial Stress

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	1793	829	0	0.84	Actively Growing	0.79	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	2366	0	30	0.99	Actively Growing	0.37	Minor Microbial Stress
07/05/2025	6	1 week post-dose	4	0	3	0.54	Viable, but not Growing	2.43	Microbial Stress
21/05/2025	8	3 weeks post-dose	1	4	7	0.24	Dying or Dormant	8.33	Lethal Microbial Stress
04/06/2025	10	5 weeks post-dose	5	0	2	0.77	Viable, but not Growing	1.08	Microbial Stress
25/06/2025	13	8 weeks post-dose	4	2	6	0.40	Dying or Dormant	2.05	Microbial Stress
23/07/2025	17	12 weeks post-dose	17	19	17	0.51	Viable, but not Growing	0.61	Minor Microbial Stress

Bioreactor C1/C2 – In-House Candidate 750 ppm/v

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	2323	1602	262	0.75	Viable, but not Growing	1.02	Microbial Stress
30/04/2025	5	**Immediately pre-dose**	2375	0	0	1.00	Actively Growing	0.35	Minor Microbial Stress
07/05/2025	6	1 week post-dose	5	1	9	0.39	Dying or Dormant	3.96	Lethal Microbial Stress
21/05/2025	8	3 weeks post-dose	1	4	8	0.20	Dying or Dormant	12.26	Lethal Stress for at Least One Day
04/06/2025	10	5 weeks post-dose	11	0	0	0.99	Actively Growing	0.61	Minor Microbial Stress
25/06/2025	13	8 weeks post-dose	1093	126	97	0.88	Actively Growing	0.27	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	972	1552	963	0.50	Viable, but not Growing	0.64	Minor Microbial Stress

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	475	118	0	0.90	Actively Growing	0.44	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	477	0	33	0.93	Actively Growing	0.45	Minor Microbial Stress
07/05/2025	6	1 week post-dose	1	3	9	0.20	Dying or Dormant	18.05	Lethal Stress for at Least One Day
21/05/2025	8	3 weeks post-dose	1	4	10	0.20	Dying or Dormant	9.82	Lethal Microbial Stress
04/06/2025	10	5 weeks post-dose	15	2	1	0.90	Actively Growing	0.70	Minor Microbial Stress
25/06/2025	13	8 weeks post-dose	14	4	9	0.59	Viable, but not Growing	0.91	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	18	19	13	0.55	Viable, but not Growing	0.44	Minor Microbial Stress

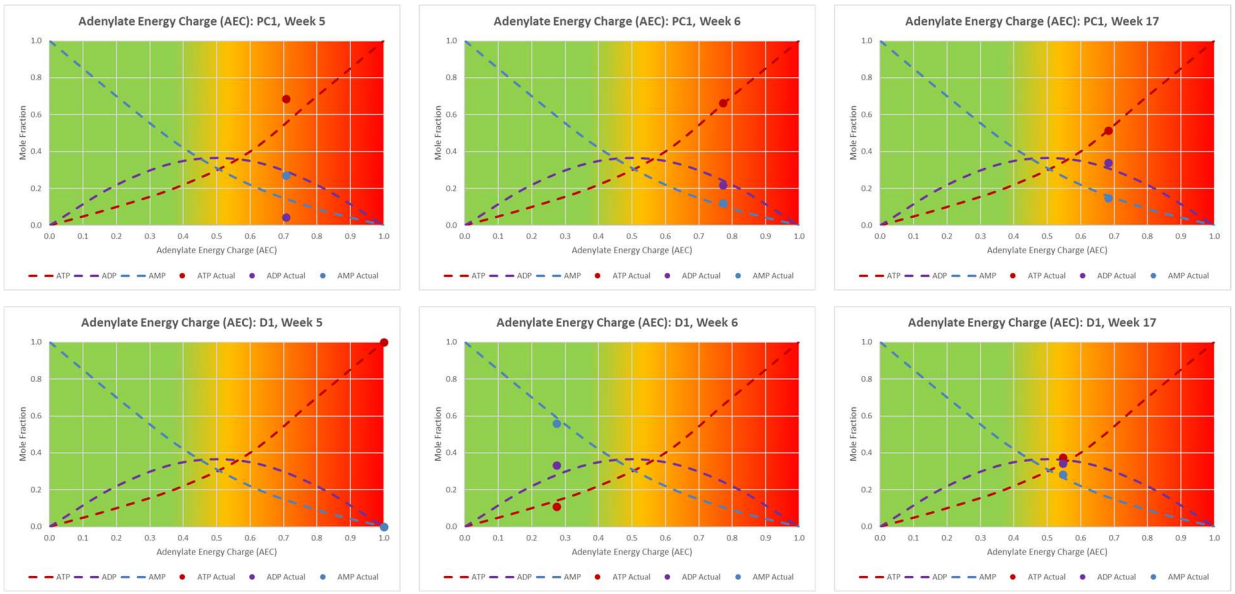
Bioreactor D1/D2 – grotan® OX 750 ppm/v

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	1688	1190	165	0.75	Viable, but not Growing	0.55	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	3687	0	0	1.00	Actively Growing	0.25	Minor Microbial Stress
07/05/2025	6	1 week post-dose	2	6	10	0.27	Dying or Dormant	10.34	Lethal Stress for at Least One Day
21/05/2025	8	3 weeks post-dose	1	4	12	0.17	Dying or Dormant	11.69	Lethal Stress for at Least One Day
04/06/2025	10	5 weeks post-dose	5	0	8	0.41	Dying or Dormant	2.92	Microbial Stress
25/06/2025	13	8 weeks post-dose	17	5	10	0.62	Viable, but not Growing	0.83	Minor Microbial Stress
23/07/2025	17	12 weeks post-dose	21	19	16	0.55	Viable, but not Growing	0.45	Minor Microbial Stress

Date	Week #	Description	pg / mL			Energy Charge (AEC)	AEC Rank	AMP Index (AMPi)	AMPi Rank
			ATP	ADP	AMP				
23/04/2025	4	1 week pre-dose	1268	414	0	0.88	Actively Growing	0.55	Minor Microbial Stress
30/04/2025	5	**Immediately pre-dose**	3576	0	0	1.00	Actively Growing	0.21	Minor Microbial Stress
07/05/2025	6	1 week post-dose	2	4	11	0.24	Dying or Dormant	11.51	Lethal Stress for at Least One Day
21/05/2025	8	3 weeks post-dose	1	4	11	0.16	Dying or Dormant	15.59	Lethal Stress for at Least One Day
04/06/2025	10	5 weeks post-dose	3	0	6	0.34	Dying or Dormant	3.75	Lethal Microbial Stress
25/06/2025	13	8 weeks post-dose	8	1	12	0.41	Dying or Dormant	1.86	Microbial Stress
23/07/2025	17	12 weeks post-dose	9	10	10	0.48	Dying or Dormant	0.74	Minor Microbial Stress

As illustrated in **Figure 6**, the AEC charts define the red zone as indicative of no metabolic stress, while the green transition zone reflects inferred stress arising from biocide application or environmental shifts. By week 17, partial stabilisation was observed from the bioreactors which had received the grotan® OX chemistry dose; however, the system did not return to the red zone. This persistence of controlled metabolic stress is widely regarded as a critical condition for effective long-term control of biogenic souring risk, supporting the strategic value of sustained, non-recoverable microbial suppression rather than short-term lethality alone.

Figure 6: Bioreactor AEC profiles for the study period (PC1 – Positive Controls (no biocide), D1 – grotan® OX 750 ppm/v).



Study Phase 4 – Microbial Enumeration

It was considered critical to include an additional culture-based assessment of microbial numbers, focused in this case on serial dilution MPN culture vials. This assessment targeted both general heterotrophic bacteria using PRD media and sulphate-reducing bacteria using MPB media. All cultures were incubated under mesophilic conditions at 30 °C, a temperature considered representative of near-wellbore conditions in a seawater flood system, which also formed the basis of the bioreactor study design.

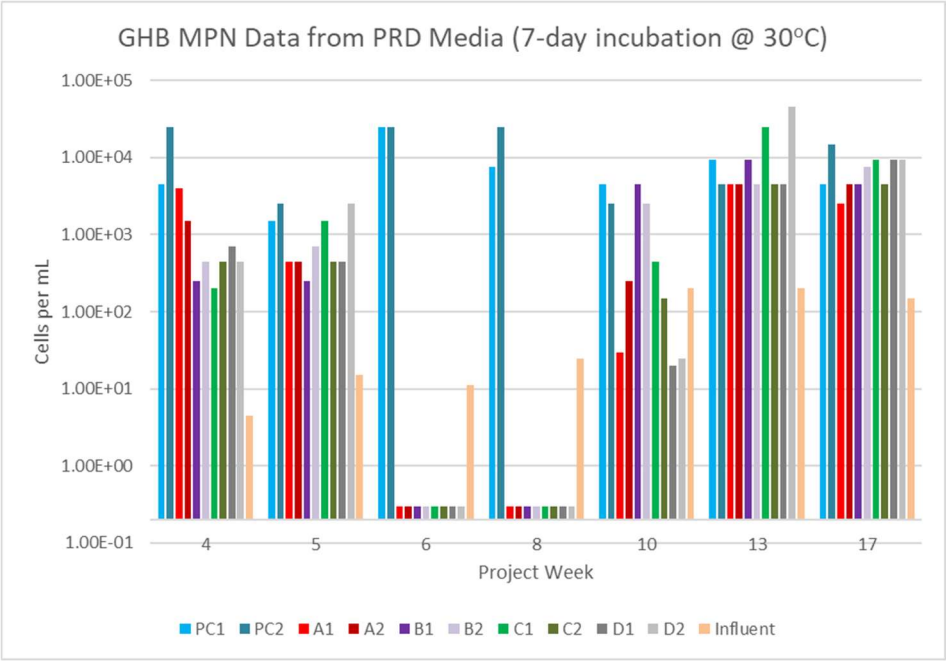
As described in **Figure 7**, significant reduction in mesophilic GHB and SRB populations was observed across all replicate bioreactor produced water samples treated with biocide in week 5, with effects persisting at the week 6 and week 8 post-treatment sampling points. During these periods, optimal control of microbial numbers was achieved, with reductions of approximately 3 log units. This level of reduction is generally aligned with accepted benchmarks for effective biocidal performance in oilfield water systems.

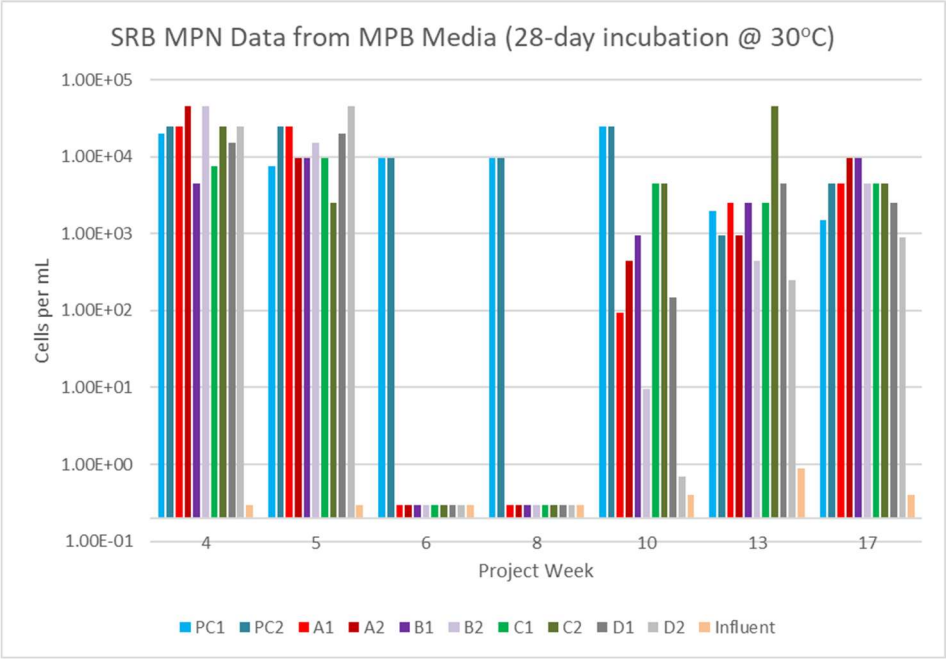
By week 10, microbial numbers were observed to recover across most treatments, consistent with the transient nature of planktonic biocide exposure. The only notable exception was observed for SRB recovery in the D2 treatment (grotan® OX at 750 ppm/v), where SRB regrowth was delayed relative to the other treatments. This observation could indicate a more persistent impact on SRB under the conditions evaluated, although full recovery was still anticipated over extended timeframes.

Overall, the data confirm that natural microbial recovery following biocide application is observed predominantly within the planktonic phase. It should be noted that these measurements do not directly represent sessile biofilm or attached biomass populations, which are often of greater operational relevance in field systems.

Consequently, while culture-based planktonic data provide valuable supporting evidence of biocide efficacy, they should be interpreted in conjunction with biofilm-focused and molecular analyses for a more comprehensive assessment of long-term microbial control.

Figure 7: Bioreactor serial dilution MPN microbial enumeration profiles for the study period (PC1 – Positive Controls (no biocide), D1 – grotan® OX 750 ppm/v).





Study Phase 5 – Molecular Review of Microbial Consortia

Sessile cultures were recovered from the initial inoculum and at the end of the study from four selected bioreactors for the purpose of molecular DNA extraction and subsequent analysis. These samples were used for bioinformatics interpretation and detailed microbial community diversity assessment. The focus on sessile material was intended to characterise attached biomass more representative of biofilm-associated populations within the bioreactor system. This approach complements planktonic culture-based data by providing deeper insight into microbial structure, diversity and potential functional shifts occurring in response to biocide exposure over the study duration.

It is commonly known that molecular data cannot differentiate between living and dead cells; therefore, the resulting datasets must be interpreted cautiously. They should be considered alongside microbial load assessments and metabolic stress parameters described in previous sections, ensuring that data interpretation reflects both viable activity and microbial load contributions to the overall microbial profile.

Initial review of the Next Generation Sequencing (NGS) dataset highlighted the following trends:

- Hydrocarbon-degrading organisms (e.g., *Alcanivorax*, *Marinobacter*, *Cycloclasticus*) were among the most abundant groups across multiple samples, confirming strong selective pressure from hydrocarbon presence.
- Sulphate-reducing and sulphur-cycling organisms (*Desulfotignum*, *Desulfofustis*, *Desulfuromusa*, *Dethiosulfatibacter*) were prominent, particularly samples PC1 (Control), A1 (Glutaraldehyde), and B1 (THPS), indicating risk for reservoir souring and sulphide-driven corrosion.
- Fermenters and acid producers (notably *Fusibacter* and *Halanaerobium*) were enriched in multiple samples, suggesting strong potential for acid generation and MIC acceleration.

When viewed alongside the NGS metabolic group distributions, quantitative polymerase chain reaction (qPCR) enumeration helped to contextualise which microbial guilds dominate the biomass. For instance, this technique could be used to confirm biomass presence in the starting core inoculum with NGS resolving it further into a hydrocarbon-degrader and sulphur-cycler dominated community. Further, the strong qPCR signals for the PC1 (Control) core sample aligned with NGS results showing enrichment of sulphate reducers, hydrocarbon degraders, and fermenters, suggesting that the biomass quantified is functionally relevant to MIC and souring risk.

We can summarise the microbial community structure differences as per below, in **Table 2** and in **Figure 8**.

- The **inoculum** established a worst-case scenario, showing how diverse microbial groups expand under nutrient-rich, uncontrolled conditions. Its dominance by fermenters,

sulphidogens, and hydrocarbon degraders reflected an environment primed for rapid biofilm development and reservoir souring if left unmanaged.

- The **PC1 control** demonstrated the consequences of hydrocarbon enrichment: microbial biomass expands exponentially, with hydrocarbon degraders (e.g., *Marinobacter*, *Alcanivorax*) driving secondary metabolism in the hydrocarbon-containing inoculum source. This fuels sulphur oxidisers, reducers, and sulphidogens, generating conditions for both severe souring and multi-pathway corrosion. The complexity and redundancy of PC1 highlighted the resilience of these microbial consortia.
- **Glutaraldehyde (A1)** partially reduces microbial loads but failed to eliminate key functional species under the defined experimental conditions. Hydrocarbon degraders persisted at high levels, while fermenters and manganese oxidisers sustained corrosion potential. Souring was moderated compared to PC1, but redundancy ensured continued activity. This reflected an incomplete control strategy with significant MIC risks.
- **THPS (B1)** exerts pressure on some groups, though leaves behind a competent anaerobic, reservoir-type community including SRB, fermenters and methanogens. This configuration can be especially problematic, as it reproduces reservoir-like souring niches. Thiosulphate reducers and methanogens indicated ongoing sulphide and acid generation, presenting a potential dual corrosion–souring threat.
- **grotan® OX (D1)** achieved stronger suppression of microbial activity relative to PC1. While hydrocarbon degraders and SRB persist, their populations (and relative abundances) are substantially reduced, with less dominance of fermenters and sulphidogens in the community dynamics. The risk profile indicated a degree of controlled souring and moderated corrosion pathways, though localised biofilm-driven MIC remained possible. Overall, grotan® OX supported better long-term control with reduced functional redundancy under the experimental conditions.

Figure 8: Bioreactor sessile deposits - Next Generation Sequencing Summary - Microbial Species Distribution (Inoculum, PC1 – Positive Control (no biocide), A1 – Glutaraldehyde 750ppm/v, B1 – THPS 750ppm/v, D1 – grotan® OX 750 ppm/v).

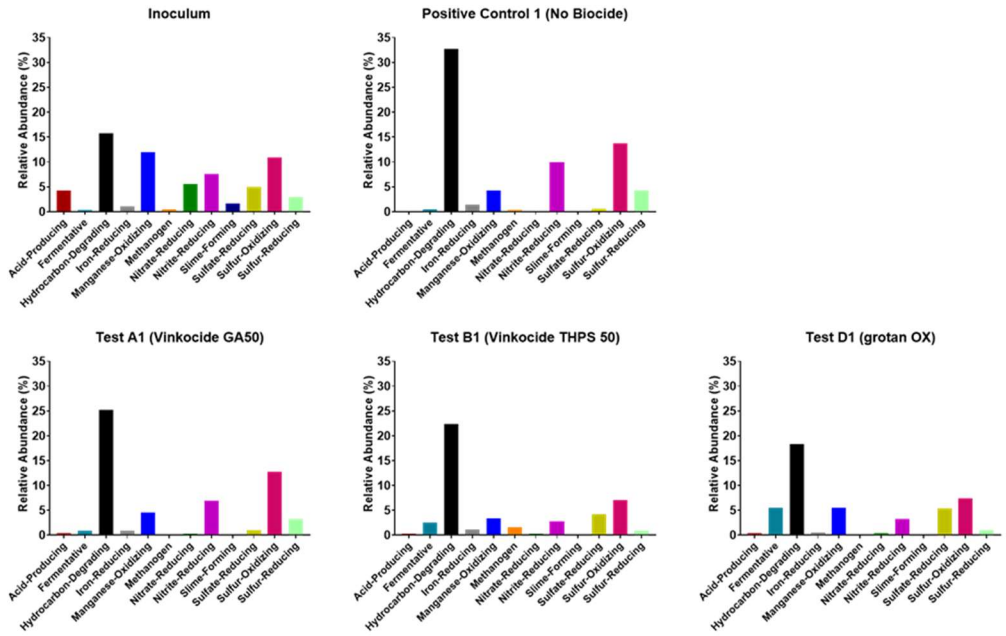


Table 2: Bioreactor conditions vs. Molecular review of the microbial consortia (PC1/2 – Positive Controls (no biocide), A1/A2 – Glutaraldehyde 750ppm/v, B1/B2 – THPS 750ppm/v, C1/C2 – In House Candidate 750 ppm/v, D1/D2 – grotan® OX 750 ppm/v).

Condition	qPCR Biomass (Total Loading)	Dominant Species	Signature Genera	Risk Profile	Interpretation
Inoculum	Very high (35–129M per group)	Fermenters, SRB, degraders	*Marinobacter, Halanaerobium, Fusibacter*	Expansion potential if uncontrolled	Worst-case microbial load
PC1 Control	Extremely high (>2.2B degraders)	Degraders, sulphur oxidizers, sulphur reducers, nitrite reducers	*Marinobacter, Alcanivorax, Desulfotignum*	Severe souring + multi-pathway corrosion	Hydrocarbon-fuelled microbial hub
A1 Glutaraldehyde	High but reduced (~943M degraders)	Degraders, fermenters, Mn oxidizers	*Fusibacter, Alteromonas*	Deposit-driven MIC, moderated souring	Partial control, resilient species remain
B1 THPS	Substantial (~699M degraders, 129M SRB)	Degraders, SRB, fermenters, methanogens	*Desulfotignum, Methanolobus, Halanaerobium*	Reservoir-type souring, MIC, methanogenesis	Risk of anaerobic activity, resilient species remain
D1 grotan® OX	Moderate (259M degraders, 76M SRB)	Degraders, sulphur cyclers, fermenters	*Marinobacter, Alcanivorax, Desulfuromusa*	Controlled souring + moderated corrosion	Stronger suppression, lower redundancy

Study Conclusions / Operational Value

The extended bioreactor study under simulated field conditions demonstrates the high operational value and long-term effectiveness of grotan® OX for biogenic souring control. Compared to both established generic biocides and in-house candidate, grotan® OX delivered:

- Superior metabolic suppression and microbial stress indications sustained for over 12 weeks and beyond
- Substantial reduction in viable biogenic hydrogen sulphide formers
- Minimal to no biogenic H_2S recovery or evolution
- Profound community disruption, verified via multiple lines of evidence

These attributes highlight the strong potential of grotan® OX as a reliable, long-acting preservative biocide solution for both remediation and prevention of biogenic souring in oilfield environments. Its performance advantages are particularly relevant in reservoir systems with long residence times, high-pressure injection operations, or restricted accessibility where frequent chemical dosing is not feasible.

The promising laboratory findings will now be progressed into controlled field trials, particularly in seawater and produced water injection systems with documented souring challenges. These trials need to assess both short-term efficacies, such as initial microbial kill, and long-term performance under operating conditions. Parallel monitoring of microbial activity, sulphide levels, and overall system integrity will be essential to ensure reliable laboratory-to-field translation.

Pilot Field Trial Adaptations / Strategy for Deployment

For any upscaling of laboratory-developed outputs to field application, operational demands and the associated shifts in reservoir matrix flow dynamics must be fully understood and proactively managed. Laboratory performance alone is insufficient; the interaction between injected chemistry, reservoir heterogeneity, and evolving waterflood conditions ultimately governs field success.

The studied chemistry has therefore been introduced through a phased deployment into a mature waterflood system in Malaysia. This staged approach is designed to minimise operational risk while allowing progressive assessment of waterflood response as the biocide programme transitions from the incumbent chemistry to grotan® OX.

During Month 1, grotan® OX is applied in every fourth treatment. In Month 2, the frequency increases to every third treatment, followed by every second treatment in Month 3. From Month 4 onward, the programme transitions fully to a new weekly batch biocide strategy focused exclusively on grotan® OX chemistry.

This managed and gradual substitution allows differentiation between short-term system stabilisation effects and longer-term reservoir-scale responses.

Reservoir communication times are estimated around 6 months, depending on the selected injector–producer pairs and prevailing sweep efficiency. As a result, first meaningful responses—covering injectivity, souring indicators, and produced water microbiology—are expected to be fully evaluated by the end of 2026, providing a robust basis for long-term programme optimisation. A robust surveillance programme is in place to truly evaluate and understand the expected operational value.

Once field validation proves successful, a scale-up and deployment strategy would be developed. This would involve creating system-specific application guidelines, integrating the solution into existing chemical treatment programs, and ensuring alignment with operator health, safety, and environmental requirements.

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