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Development of High-Specificity Primers for the Detection of Sulfate-Reducing Bacteria from the Petroleum Environment Through qPCR

Beatriz Marques Lobato

beatrizlobato@usp.br

Universidade de São Paulo

Caio Perez Gomes

Federal University of São Paulo

Maria Filomena de Andrade Rodrigues

Instituto de Pesquisas Tecnológicas

Cintia Paula Jandre Rua

Instituto de Pesquisas Tecnológicas

Patricia Léo

Universidade de São Paulo

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Abstract

Microbiologically influenced corrosion (MIC) associated with sulfate-reducing bacteria (SRB) represents a persistent challenge for the oil and gas industry, particularly in environments where conventional monitoring tools fail to capture microbial complexity. In this work, we developed and experimentally validated a set of highly specific primers for the detection of SRB in industrial samples using quantitative PCR (qPCR). Primer design was guided by metataxonomic analyses of produced water from oilfields, targeting conserved regions of the *dsrB* gene. Validation assays were performed using microcosms containing both planktonic and sessile communities derived from pure cultures and mixed consortia. The primers showed high amplification efficiency, specificity, and sensitivity across all tested conditions. By enabling rapid and reliable detection of SRB in complex matrices, this approach provides a practical alternative to culture-dependent methods and supports more accurate microbiological monitoring in corrosion-prone industrial systems.

1 INTRODUCTION

Microbiologically influenced corrosion (MIC) is the degradation of metal caused, partially or entirely, by microbial activity [1]. In oil and gas production systems, sulfate-reducing bacteria (SRB) are consistently reported as key contributors to this process due to their ability to reduce sulfate to sulfide, generating corrosive by-products that destabilize protective metal films [2]. The consequences of MIC extend beyond material degradation, often resulting in unplanned shutdowns, product losses, and environmental damage caused by leaks [3, 4]. From an economic perspective, MIC represents a substantial burden for the oil and gas industry, with estimated annual costs ranging from $3 to $7 billion, corresponding to nearly 20% of total corrosion-related expenditures [5, 6, 7].

Detecting and quantifying microbial populations in oil production systems remains challenging when based solely on culture-dependent approaches. Methods such as the Most Probable Number (MPN) are still routinely applied in the oil industry for monitoring bacterial presence in pipelines [8, any progress], despite well-recognized limitations. A major constraint is the inability of these methods to capture the true diversity of the original microbial community, as a substantial fraction of environmental microorganisms fails to grow under laboratory conditions [9]. This issue is particularly relevant for anaerobic microorganisms, which dominate many oilfield environments. Exposure to oxygen during sampling and handling can impair their viability, leading to cell inactivation or death before cultivation is attempted [10]. In addition to these biological constraints, MPN-based analyses are operationally demanding. The method requires extended incubation periods, daily monitoring of multiple dilution series, and indirect estimation of cell numbers based on growth patterns [11]. For sulfate-reducing bacteria, incubation times may extend up to 28 days before results can be obtained [8, 12]. Together, these factors limit the reliability of culture-based assessments and may result in an underrepresentation of microbial populations relevant to MIC, ultimately affecting risk evaluation and mitigation strategies [8].

Culture-independent approaches based on molecular biology have been increasingly adopted for the investigation of microbial communities associated with MIC, as they bypass many of the constraints inherent to cultivation-based methods [13, 14]. Among these, quantitative PCR has been widely applied for the detection and quantification of sulfate-reducing bacteria in industrial systems [15]. However, most qPCR assays described to date were developed using isolated strains as reference material. Primer design has therefore been largely based on genes derived from pure cultures, frequently targeting specific taxa such as *Desulfovibrio vulgaris* or members of the genus *Desulfotomaculum* [16, 17, 18]. While these assays perform well under controlled conditions, their applicability to complex industrial samples is limited. Oilfield environments typically harbor diverse SRB assemblages, and primers designed from a narrow set of reference strains may fail to detect phylogenetically distant, yet functionally relevant, populations. As a result, molecular monitoring strategies based solely on isolated representatives may provide an incomplete picture of SRB distribution and activity in pipelines and produced water systems.

Based on this approach, the present study describes the design of qPCR primers targeting the *dsrB* gene using metataxonomic data from a petroleum-associated sample, followed by experimental validation of primer specificity and performance. The experimental procedures adopted for sample processing, molecular analyses, and validation assays are detailed in the following sections.

2 EXPERIMENTAL PROCEDURES

2.1 Sample Collection and Processing

A produced water sample was collected from the oil-water separator of an offshore production well located in the Campos Basin (Rio de Janeiro State, Brazil). The field has been in operation since 2005 and presents a documented history of biogenic acidification. The sample was provided by Centro de Pesquisas e Desenvolvimento Leopoldo Américo Miguez de Mello (CENPES).

A total volume of 100 mL was filtered through 0.22 µm pore-size nitrocellulose membranes (47 mm diameter; Millipore, São Paulo, Brazil). After filtration, membranes were rinsed with sterile PBS, aseptically sectioned, and transferred to 2 mL microtubes. Samples were stored at -20°C until DNA extraction.

2.2 DNA Extraction

Total DNA from planktonic microbial communities was extracted using a CTAB-based protocol adapted from [19]. The membrane-containing microtubes received 600 µL of CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA 20 mM, Tris-HCl pH 8.0 100 mM) and 1.5 µL of Proteinase K (20 mg/mL) were added, followed by incubation at 55°C for 2 h, with occasional vortexing for homogenization. Following lysis, samples were extracted twice with 600 µL of chloroform:isoamyl alcohol (24:1), mixed vigorously for 3 min, and centrifuged at 14,000 rpm for 10 min. The supernatant was transferred to a new sterile microtube, and 10% 3M sodium acetate and 2.5 volumes of chilled isopropanol were added, followed by precipitation at

-20°C overnight. After, the microtubes were centrifuged at 14,000 rpm for 30 min, and the supernatant was discarded. Pellets were washed twice with 500 µL of chilled 70% ethanol and centrifuged at 14,000 rpm for 10 min. After complete drying, DNA was resuspended in 50 µL of sterile Milli-Q water and stored at 4°C until use.

DNA concentration was determined using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, USA), and integrity was verified by 1% (w/v) agarose gel electrophoresis.

The CTAB-based protocol was selected due to its suitability for environmental samples containing high salt concentrations and petroleum-derived compounds. Such matrices are known to interfere with enzymatic amplification when residual inhibitors are present. Compared to commercial extraction kits, the CTAB method allows greater flexibility in buffer composition and washing steps, which is advantageous when processing petroleum-associated samples [19].

2.3 Amplification and analysis of the 16S rRNA gene

Bacterial community profiling was performed by amplification of the V3-V4 region of the 16S rRNA gene using primers 8F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 1492R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) with Illumina overhang adapters [20]. PCR reactions were carried out in a ProFlex PCR System (Thermo Scientific). Each 20 µL reaction contained 250 nM of each primer, 10 µL of OneTaq® 2X Master Mix (New England BioLabs, Ipswich, MA, USA), 7.4 µL of nuclease-free water, and 1 µL of template DNA.

The thermal cycling conditions were as follows: initial denaturation at 95°C for 30 s, followed by 30 cycles of 95°C for 15 s, 55°C for 20 s, and 68°C for 30 s, with a final extension at 68°C for 5 min, and holding at 4°C. The results were visualized by 1% (w/v) agarose gel electrophoresis. The amplified products were then submitted for amplicon sequencing using Illumina MiSeq platform (Illumina, San Diego, CA, USA).

2.4 Diversity analysis

Sequencing data were processed following established workflows described by [21] [19]. Quality filtering and trimming were performed using SolexaQA+, and paired-end reads were merged with PANDA. Chimeric sequences were identified and removed using UCHIME. OTU clustering was conducted with Swarm, and taxonomic assignment was performed in Mothur using the SILVA (v1.32) database.

Taxonomic abundance tables were generated and used for visualization of community composition. Diversity indices and richness estimates were calculated at the genus level using LibreOffice Calc (v. 7.3.2.2). Functional potential was inferred using FAPROTAX v1.2.6 based on taxonomic profiles [22].

2.5 Design of Primers for the DsrB Gene Region

Primer design targeted conserved regions of the *dsrB* gene identified from metataxonomic data obtained from produced water. Representative sequences from SRB genera detected at higher relative abundance

(*Desulfonauticus*, *Desulfoplanes*, *Desulfothermus*, *Desulfovibrio*, and *Desulfococcus*) were selected for alignment.

Nucleotide sequences of the *dsrAB* operon corresponding to these genera were retrieved from GenBank public databases. Multiple sequence alignments were performed using Geneious R11.1, employing the MUSCLE algorithm for accurate detection of conserved regions within the *dsrB* subunit.

Candidate regions of conservation within dsrB were then manually inspected to define potential primer-binding sites. Specificity and coverage were evaluated by including a broad taxonomic representation of known SRB sequences during the alignment process. Primers were designed to amplify a region of approximately 198 bp, which is compatible with downstream qPCR and sequencing applications.

Thermodynamic parameters of the primer candidates, melting temperature (Tm), GC content, self-dimer and heterodimer formation potential, and hairpin structures, were calculated using IDT OligoAnalyzer Tool (https://www.idtdna.com/pages/tools/oligoanalyzer). Primers showing potential secondary structures or unfavorable interactions were excluded from further testing.

To evaluate the specificity of the designed primers, the predicted amplicon sequences were subjected to BLASTn searches against the NCBI GenBank nucleotide collection to confirm homology exclusively with *dsrB* sequences from SRB taxa. Additionally, in silico PCR was performed using the UCSC In-Silico PCR tool to confirm that no off-target amplification occurred with closely related non-SRB microorganisms.

The final primer pair, named BRS-DsrB-F and BRS-DsrB-R, was selected based on its coverage, specificity, and favorable thermodynamic profile. These primers were then synthesized and validated experimentally with DNA templates derived from environmental samples and positive SRB controls. Their sequences and properties are detailed in Table 1.

2.6 Quantitative PCR Optimization

qPCR reaction was performed using a StepOnePlus™ Real-Time PCR System (Thermo Scientific). Each 20 µL reaction contained 150 nM of each primer, 10 µL of PowerTrack SYBR Green (Thermo Scientific) with the internal passive reference dye ROX, 7.4 µL of DEPC-treated water, and 1 µL of extracted DNA. Each sample was amplified in triplicate.

The standard thermal cycling conditions were as follows: Initial denaturation at 95°C for 2 min. 40 cycles of 95°C for 15 s (denaturation) and 60°C for 30 s (annealing/extension). Fluorescent signal collection was enabled during the extension step.

Ct values were exported for downstream analysis, and amplification performance was evaluated based on reproducibility across technical replicates.

A tenfold serial dilution regression analysis was used to determine the limits of detection (LoD), starting with 100 ng/µL and ending at 0.01 ng/µL of *Desulfovibrio desulfuricans* gDNA. The LoD for each primer

was identified based on the presence or absence of a detectable signal. All standard curves were performed in triplicate.

2.7 Bacterial Culture

For the enrichment of the culture, 5 mL of the water sample were inoculated into 45 mL of a modified Postgate C medium ($Na_3C_6H_5O_7$ 0.3 g/L, yeast extract 1.0 g/L, NH_4Cl 1.0 g/L, Na_2SO_4 4.6 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.06 g/L, $CaCl_2 \cdot 6H_2O$ 0.06 g/L, $FeSO_4 \cdot 7H2O$

0.004 g/L, sodium lactate 50% 9.4 mL/L, resazurin 0.025% w/v 4.0 mL/L, pH 7.6) [23]. The Postgate C medium is a classical formulation specifically designed for the selective enrichment and maintenance of SRB inocula. In this study, it was modified to better mimic the physicochemical characteristics of the environment, including adjustments of NaCl concentration to match the salinity of the formation water and the reduction of $FeSO_4$ concentration to minimize iron sulfide precipitation under saline conditions.

To ensure anaerobic conditions, the medium was prepared and dispensed under anoxic atmosphere using N_2 gas flushing for 5 min, and the penicillin bottles were immediately sealed with butyl rubber stoppers and aluminum crimps. The presence of resazurin served as a redox indicator to confirm anaerobiosis. Cultures were incubated at 30°C for 48 h, until visual evidence of SRB activity was observed, characterized by the blackening of the medium due to H_2S production [23, 24]. The resulting enrichment was later used as a mixed-culture inoculum for microcosm assays.

2.8 Detection of SRB DNA in Comparative Microcosm Assays

A field validation assay was conducted, simulating microcosms containing test specimens made of AISI 1020 carbon steel in a rectangular shape with approximate dimensions of 30 mm × 10 mm × 1 mm. The microcosms were prepared using four SRB inocula, which included strains of *D. desulfuricans* (ATCC 29577), *D. vulgaris* (ATCC 7795), *D. nigrificans* (ATCC 19998) and a mixed culture isolated from the produced water of the offshore platform, all tested in triplicate. To maintain anaerobiosis during the 30-day incubation, all bottles were purged with N_2 for 5 min prior to sealing with butyl rubber stoppers and aluminum crimps, ensuring oxygen-free headspace. The incubation was carried out at optimal temperatures for each strain (30°C, 37°C, 55°C, and 30°C respectively) [24].

After this period, the penicillin vials were opened in a hood due to the production of H_2S by the bacteria. After incubation, carbon steel specimens were aseptically removed and transferred to a sterile tube containing reducing saline solution, which was sonicated for 20 min to remove the biofilm. The resulting solutions were then filtered using a nitrocellulose membrane with a pore size of 0.22 µm and transferred to 2 mL microtubes containing 600 µL of 2% CTAB buffer for subsequent DNA extraction.

The same procedure was applied to the planktonic phase. A volume of 20 mL from each microcosm was filtered through 0.22 µm nitrocellulose membranes and processed for DNA extraction as described

above.

3 RESULTS AND DISCUSSION

3.1 Bacterial Diversity in Produced Water

The 16S rDNA-based metataxonomic analysis revealed the structure of the microbial community present in the produced water sample collected from a pipeline with a known history of variable corrosion intensity. Rather than establishing direct causality, the analysis aimed to contextualize the dominant microbial groups potentially associated with corrosion-prone conditions.

A total of 6,640 reads were taxonomically assigned, with the community dominated by members of the phyla *Firmicutes*, *Proteobacteria*, and *Halanaerobiaeota*. This taxonomic profile aligns with previous observations from oilfield environments characterized by high salinity and low oxygen availability [25, 26]. At the genus level, *Desulfonauticus* (51.6%), *Desulfoplanes* (11.4%), and *Desulfothermus* (5.2%) were the most abundant taxa, together with representatives of the order *Desulfovibrionales* (3.8%). All of these groups are recognized sulfate-reducing bacteria (SRB) with established relevance to MIC.

Alpha diversity metrics suggested a moderately structured community, characterized by the dominance of a limited number of taxa alongside a broader set of low-abundance organisms. Beta diversity analysis based on Bray-Curtis dissimilarity indicated clustering driven primarily by SRB-dominated profiles, supporting the relevance of these groups in the sampled system.

Taken together, the dominance of SRB-related taxa reinforces the need for monitoring approaches that target functional genes directly linked to sulfate reduction rather than relying solely on taxonomic markers.

3.2 Primer Design and Experimental Validation

Alignment of *dsrAB* sequences obtained from the metataxonomic dataset was performed using Geneious R11.1 and compared with reference sequences available in GenBank. Based on the identification of conserved regions within the *dsrB* gene, a primer pair (BRS-DsrB-F and BRS-DsrB-R) was designed to amplify a 198 bp fragment (Table 1, Fig. 3).

Table 1
Oligonucleotides used for real-time polymerase chain reaction (qPCR). (**F**) and (**R**), direct and reverse primers, respectively.

Primer name	Sequence 5’ – 3’	Primer binding site	Product size (bp)
DsrB-F	TTG TGG GTA TCC ACC GCA A	187–202	198
DsrB-R	GGC ACA TGG TGT AGC AGT T	391–408	

In silico analyses indicated that the primer pair is compatible with a broad range of SRB taxa and does not match dissimilatory sulfite reductase genes from non-SRB organisms.

PCR amplification was observed for all tested SRB templates, including reference strains and the mixed culture derived from produced water, while no amplification was detected in negative controls. These results support the applicability of the primer set for detecting *dsrB* in environmental samples.

A careful selection of genetic markers is crucial to ensuring the accurate identification and quantification in environmental samples. Although *dsrA* has been widely used as a molecular marker for sulfate-reducing microorganisms, sequence variability across SRB taxa can affect primer performance. During alignment analyses, the *dsrB* region showed a higher degree of conservation among the species examined, which informed the decision to target this subunit for primer design (Fig. 4). The primer set showed consistent amplification profiles and clean dissociation curves, with no evidence of nonspecific products under the conditions tested. No amplification was observed for non-SRB reference strains, indicating a high degree of specificity.

The qPCR primers developed in this study demonstrated effectiveness in the specific detection and quantification of SRB in water samples from oil fields. This method proved to be highly sensitive, enabling high-throughput bacterial analysis in environmental samples. During qPCR analysis, no secondary peaks were observed in the dissociation curve, indicating the absence of nonspecific amplification. The specificity of the primers was successfully validated, resulting in no false positives and highlighting their selective hybridization capability exclusively with the tested SRB species, while excluding unrelated species (Figs. 4). This reliable and accurate method constitutes an essential tool for monitoring and studying microbial diversity in industrial wastewater.

The primer design process was based on multiple alignments of reference sequences from *Desulfonauticus*, *Desulfoplanes*, *Desulfothermus*, *Desulfovibrio*, and *Desulfococcus*, all of which were identified in the metataxonomic dataset.

Although previous studies have described primers targeting *dsrAB* genes [27, 17, 28] this is the first report of primers specifically optimized for detection of *dsrB* in SRB communities enriched in produced water from Brazilian oilfields. When compared to existing primers, the ones developed in this study demonstrated greater coverage of halophilic and thermophilic SRB taxa commonly overlooked in earlier designs.

3.3 qPCR Efficiency and Reaction Standardization

The establishment of a robust and reproducible qPCR protocol is critical for accurate quantification of SRB in environmental samples. Primer concentrations were optimized empirically, with 100 nM yielding the most stable amplification profiles when tested with *D. desulfuricans* genomic DNA.

The standard curve exhibited strong linearity ($R^2 = 0.998$) (Fig. 5) across the tested dilution range, with an amplification efficiency of 99%, consistent with expected performance for SYBR Green-based assays.

Maintaining consistent qPCR efficiency across all template concentrations is essential for quantitative precision. Deviations in slope beyond the optimal range (typically − 3.1 to -3.6) can reflect suboptimal reaction conditions or primer-dimer formation. However, no such issues were observed, as evidenced by the clean melting curves and reproducibility among replicates.

3.4 Detection of SRB DNA in Comparative Microcosm Assays

After 30 days of incubation, SRB activity was indicated by the formation of black iron sulfide precipitates (FeS) in most microcosms. In contrast, no visible precipitate was observed in assays inoculated with *D. nigrificans*, although slight darkening of the medium was noted, possibly reflecting reduced metabolic activity at the incubation temperature of 55°C.

The primary objective of this experiment was to evaluate SRB proliferation under planktonic and sessile conditions using both the developed qPCR method and the conventional Most Probable Number (MPN) assay. The comparative quantification results are presented in Tables 2 and 3.

Table 2
Comparison of the results of the quantification of planktonic bacteria in the inoculum samples, first day and after 30 days of testing by qPCR and MPN methodologies. The tests were carried out in technical triplicates and the average of the results was calculated.

Planktonic samples	**qPCR (copies/mL)**	**MPN/mL**
D. desulfuricans - Inoculum	8,89E + 07	9,50E + 04
D. vulgaris - Inoculum	2,62E + 07	2,50E + 00
D. nigrificans - Inoculum	6,51E + 04	0
Produced water - Inoculum	2,11E + 04	4,50E + 05
D. desulfuricans – 1st day	5,01E + 07	2,50E + 03
D. vulgaris – 1st day	1,27E + 07	2,50E + 00
D. nigrificans – 1st day	2,60E + 02	0
Produced water – 1st day	1,48E + 03	4,50E + 03
D. desulfuricans – 30 days	2,29E + 07	2,17E + 00
D. vulgaris – 30 days	2,38E + 07	1,07E + 01
D. nigrificans – 30 days	1,51E + 03	0
Produced water – 30 days	6,14E + 04	1,15E + 03

Table 3
Comparison of the results of the quantification of sessile bacteria after 30 days of testing by qPCR and MPN methodologies. The tests were carried out in technical triplicates and the average of the results was calculated.

Sessile samples	qPCR (copies/mL)	MPN/mL
D. desulfuricans – 30 days	7,77E + 05	2,17E + 00
D. vulgaris – 30 days	1,01E + 04	7,59E + 00
D. nigrificans – 30 days	1,35E + 02	0,00E + 00
Produced water – 30 days	1,08E + 04	0,00E + 00

As shown in Table 2, qPCR consistently detected higher quantities of SRB DNA in planktonic samples than MPN, especially during the early stages of incubation. For instance, *D. vulgaris* exhibited a notable discrepancy, with qPCR detecting approximately 10^7 copies/mL while MPN reported only 2.5 MPN/mL at day 0. Similarly, in *D. desulfuricans*, qPCR detected over 10^7 copies/mL at all time points, while MPN underestimated the bacterial population, especially after 30 days.

Sessile bacterial populations recovered from carbon steel surfaces after 30 days are detailed in Table 3. Again, qPCR detected significantly higher concentrations than MPN, particularly for *D. desulfuricans* (7.77 x 10^5 copies/mL by qPCR vs. 2.17 MPN/mL). For *D. nigrificans*, both assays showed minimal to no detection, aligning with the lack of visible growth.

Given the non-normal distribution of the data, differences between qPCR and MPN results were evaluated using the Wilcoxon signed-rank test, which indicated a significant discrepancy between methods ($p < 0.000001$). While a paired t-test suggested a similar trend, the non-parametric approach was considered more appropriate for this dataset (Fig. 6).

Overall, the qPCR approach consistently detected SRB in samples where MPN yielded low or null counts, particularly for sessile populations and thermophilic strains. These findings illustrate the limitations of culture-based quantification under conditions that restrict growth and highlight the practical advantages of gene-targeted methods for monitoring SRB dynamics in industrial systems.

4 CONCLUSIONS

The results obtained indicate that qPCR, when combined with primers specifically designed for conserved regions of the *dsrB* gene, constitutes a reliable approach for detecting sulfate-reducing bacteria in industrial samples. Compared with culture-based methods such as the Most Probable Number assay, the molecular strategy proved to be faster and more sensitive, particularly in systems containing slow-growing or non-culturable populations. The consistency observed across planktonic and sessile samples highlights the suitability of this method for routine monitoring of corrosion-prone environments. Overall, the approach described here supports the incorporation of molecular diagnostics

into microbiological control programs aimed at mitigating microbiologically influenced corrosion in petroleum production systems.

Declarations

Ethical Statement

This study involved only environmental microbial samples and laboratory-based assays, without the use of human or animal subjects. Accordingly, approval from an institutional ethics committee was not required.

Conflict of interest

The authors declare no potential conflict of interests.

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Author Contribution

BML contributed to the conceptualization, methodology, investigation, validation, visualization, and resources of the study, and was responsible for writing the original draft and revising and editing the manuscript. CPG contributed to software development, data curation, formal analysis, and resources. MFAR contributed to supervision, project administration, funding acquisition, and resources. CPJR contributed to conceptualization, data curation, and resources. PL contributed to conceptualization, supervision, project administration, funding acquisition, and manuscript review and editing.

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Data Availability Statement

The data supporting the findings of this study are included in the Supplementary Material and are also available from the corresponding author upon reasonable request.

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Figures

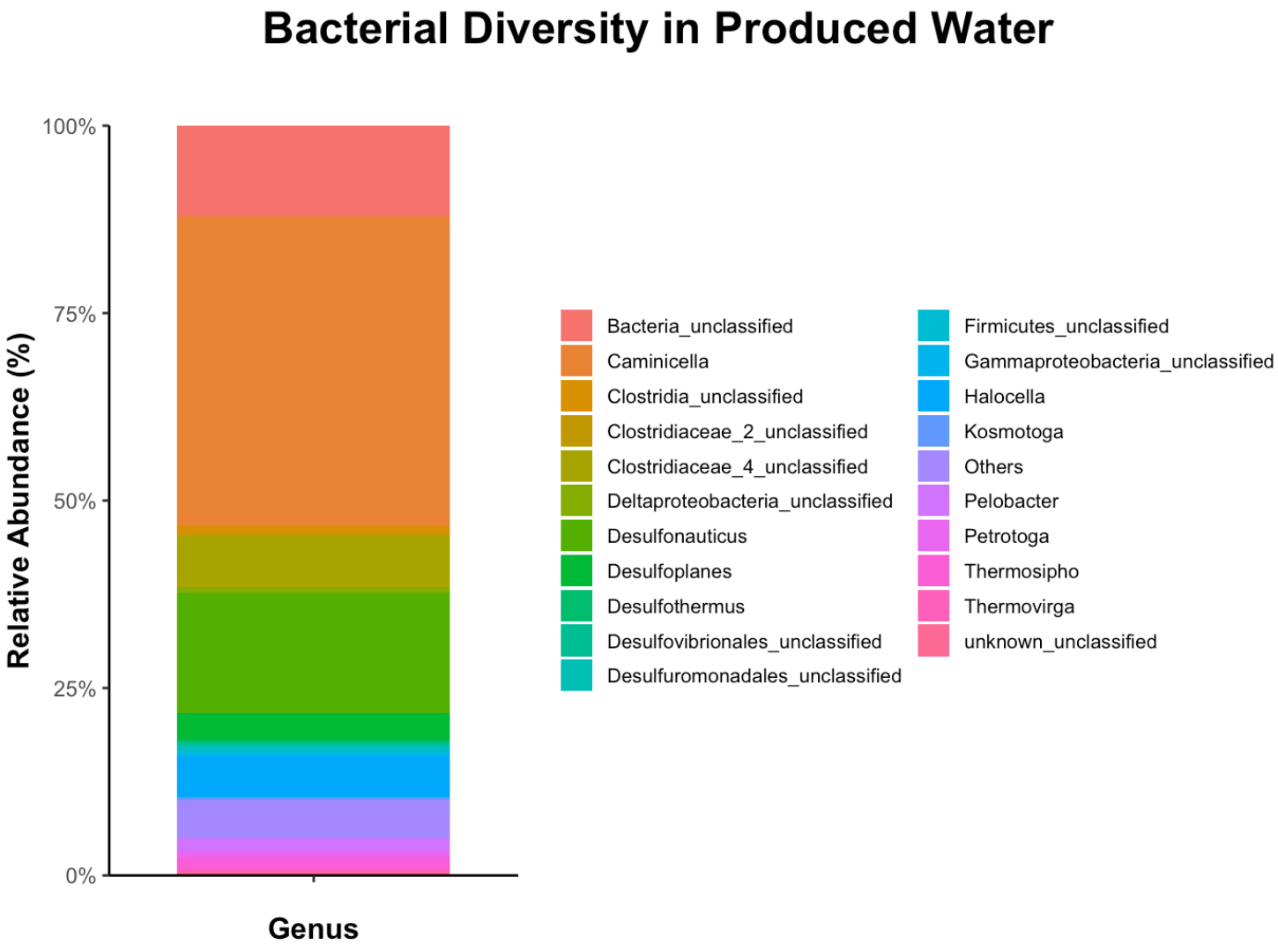


Figure 1

Relative abundance of bacterial genera identified in the produced water sample based on 16S rDNA sequencing. The figure shows the taxonomic composition at the genus level, highlighting the

predominance of Firmicutes and Proteobacteria phyla. Genera such as Caminicella, Desulfonauticus, Desulfoplanes, and Halanaerobium were among the most abundant, suggesting potential microbial contribution to the biocorrosion observed in the system.

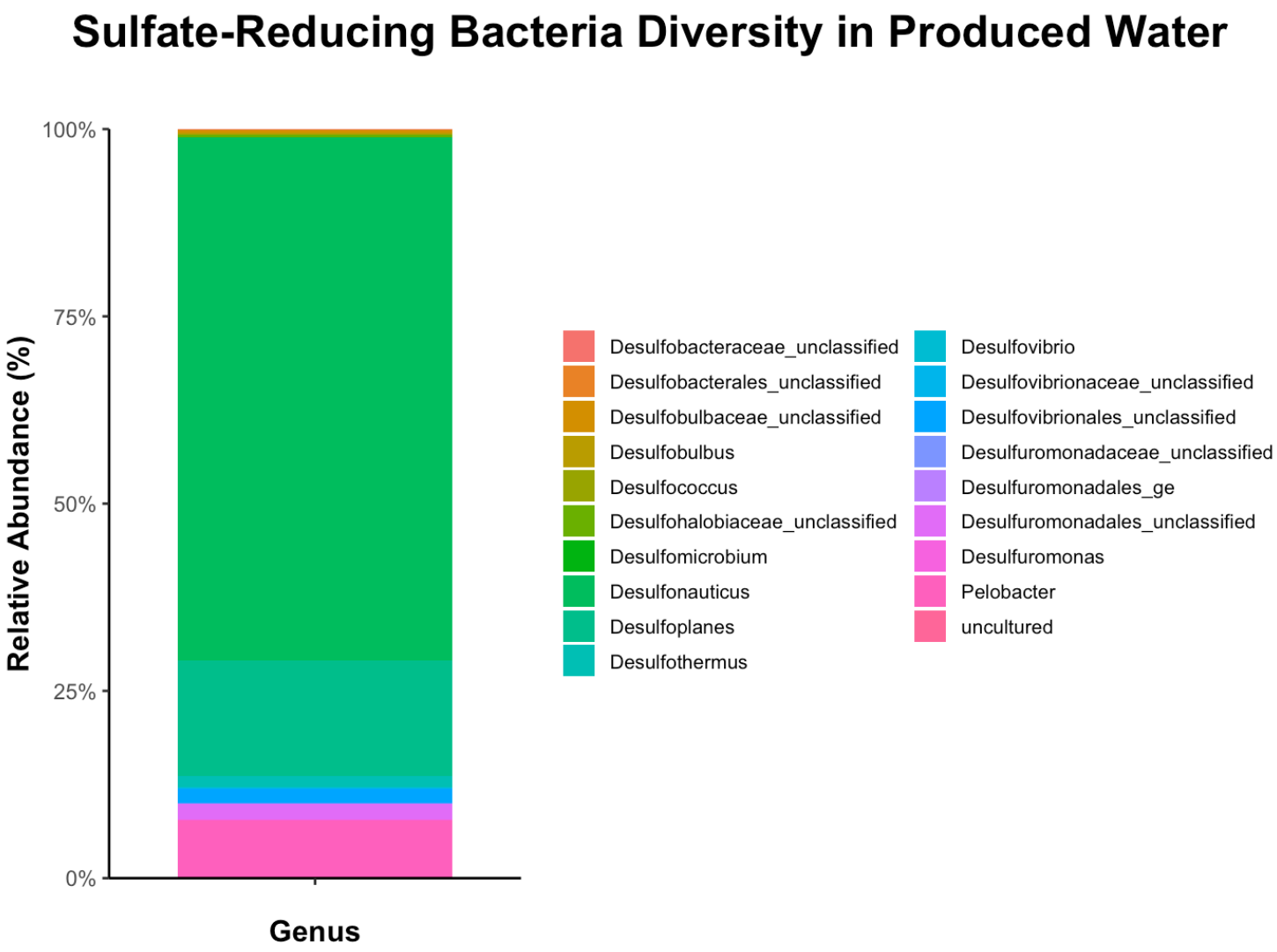


Figure 2

Relative abundance of Sulfate-reducing bacteria (SRB) genera identified in the produced water sample. SRB taxa were filtered from the overall dataset to highlight key genera implicated in microbiologically influenced corrosion (MIC). Desulfonauticus, Desulfoplanes, and Desulfothermus showed high relative abundance, corroborating their role in sulfate reduction and possible H_2S generation in oilfield environments.

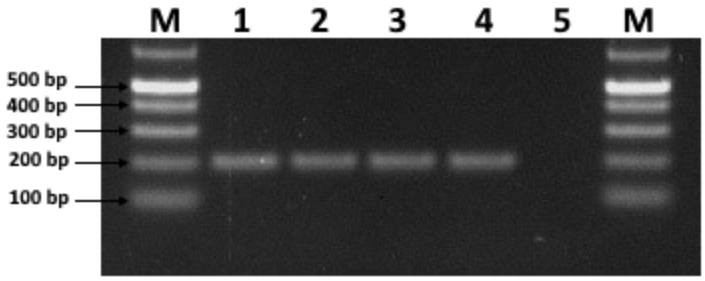


Figure 3

Agarose (2.0%) gel of real-time PCR amplification products of dsrB genes with five different samples. Lane M: Marker DNA; lane 1: D. desulfuricans; lane 2: D. vulgaris; lane 3: D. nigrificans; lane 4: Mixed culture from wastewater; lane 5: Negative control without DNA sample.

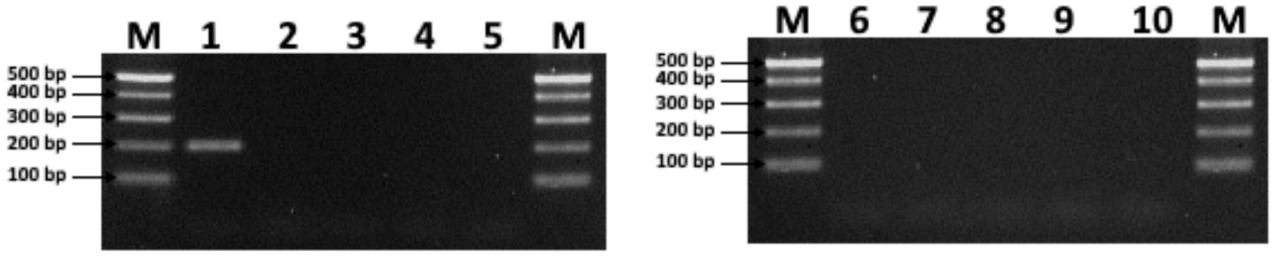


Figure 4

A. Agarose (2.0%) gel of PCR specificity test of the BRS-DsrB-F and BRS-DsrB-R primers with five different samples. Lane M—Marker DNA; lane 1 D. desulfuricans; lane 2 E. coli; lane 3 S. cholerasis; lane 4 P. aeruginosa; lane 5 S. epidermidis. **B.** Agarose (2.0%) gel of PCR specificity test of the BRS-DsrB-F and BRS-DsrB-R primers with five different samples. Lane M—Marker DNA; lane 6 S. aureus; lane 7 C. acnes; lane 8 S. gougerotii; lane 9 B. cepacia; lane 10 C. xerosis.

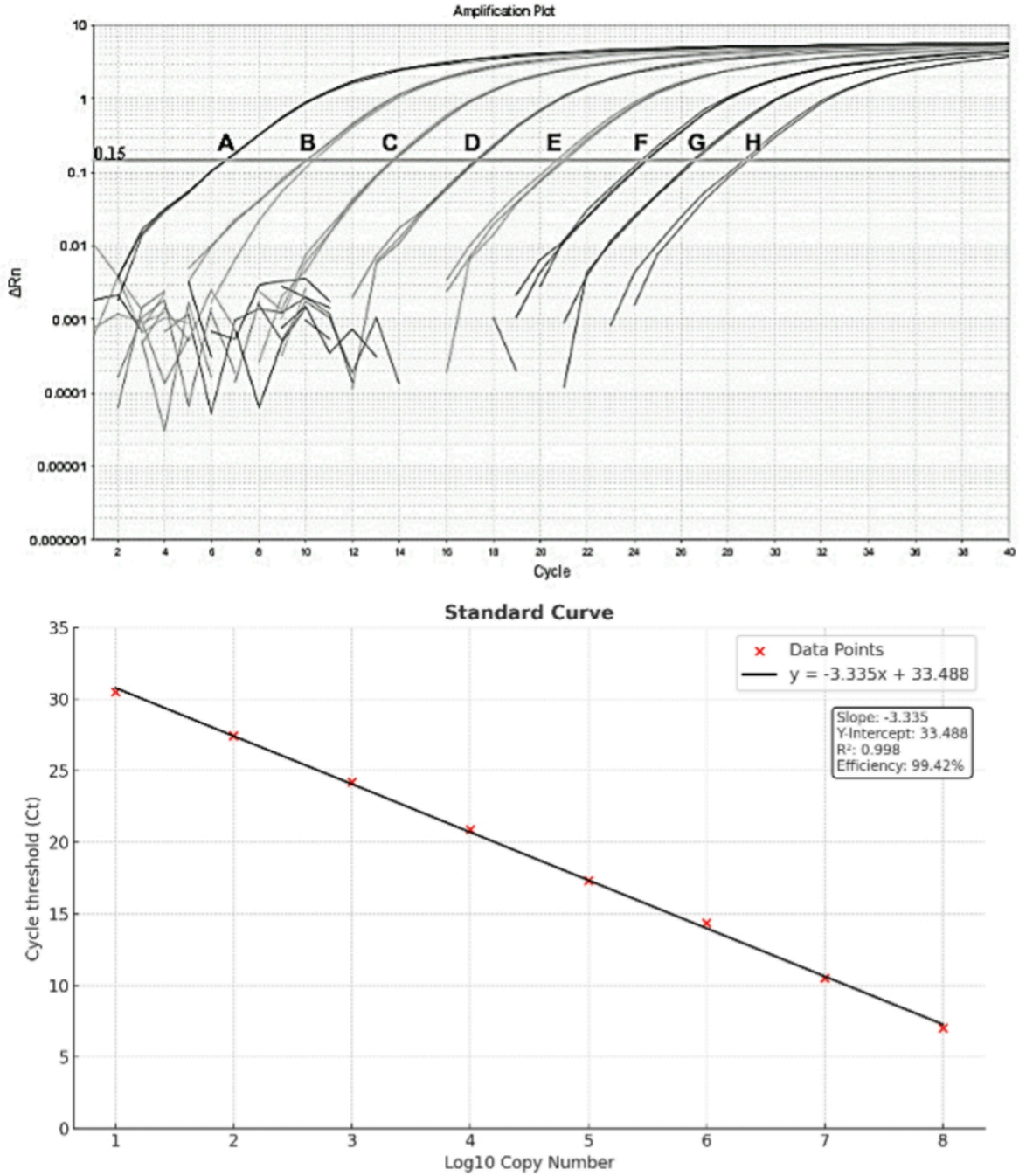


Figure 5

The standard curve was obtained by plotting the concentration of D. desulfuricans gDNA using the primer set BRS-DsrB-F/BRS-DsrB-R for the dsrB gene.

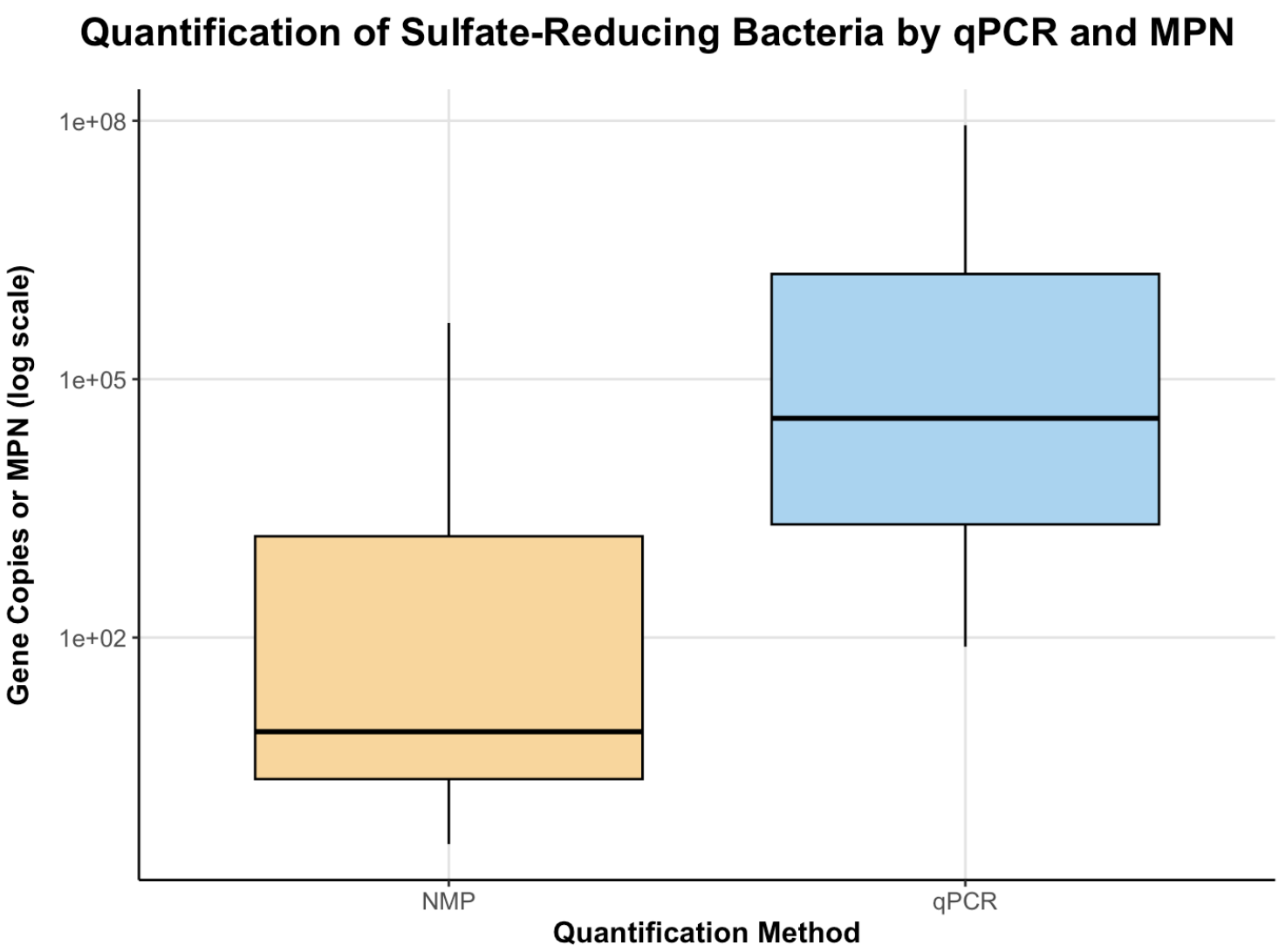


Figure 6

Distribution of SRB quantification results obtained by qPCR and MPN methods. The boxplot highlights the greater consistency and higher sensitivity of the qPCR method, which detects broader concentration ranges with less variability across replicates.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.docx