

Inhibition of X65 carbon steel corrosion by *Desulfovibrio ferrophilus* IS5 using plant-based anthraquinone

Adnan Khan
Dept. of Biological Sciences
Ohio University
Athens, OH 45701, USA

Gary Jenneman
GJ Microbial Consulting, LLC
Bartlesville, OK 74006, USA

Lingjun Xu
Dept. of Chemical & Biomolecular Eng.,
Inst. for Corrosion & Multiphase Tech.
Ohio University
Athens, OH 45701, USA

Ed Burger
EB Technologies
Dallas, TX 75205, USA

Tingyue Gu
Dept. of Chemical & Biomolecular Eng.
Inst. for Corrosion & Multiphase Tech.
Ohio University
Athens, OH 45701, USA

ABSTRACT

Sulfate reducing bacteria (SRB) biofilms are often responsible for microbiologically influenced corrosion (MIC) in oil and gas pipelines. Biocides together with pigging are employed to mitigate pipeline MIC. In this work, anthraquinone (AQ), a plant-based SRB inhibitor, was assessed as a green chemical to mitigate X65 carbon steel MIC. *Desulfovibrio ferrophilus* IS5, a highly corrosive SRB strain, was grown in enriched artificial seawater (EASW) culture medium at 28 °C. Various assays, including cell counts, biofilm imaging, X65 carbon steel weight loss measurements, and electrochemical tests were used to determine the efficacy of AQ against SRB MIC. An undetectable SRB sessile cell count and 99% decrease in weight loss were achieved by 25 ppm (w/w) AQ after 7 d of static incubation. Biofilm imaging corroborated the sessile cell count data. In comparison, 25 ppm tetrakis (hydroxymethyl) phosphonium sulfate (THPS) reduced sessile *D. ferrophilus* cells below detection limits and 99% decrease in weight loss, similar to AQ, but unlike AQ, THPS also strongly decreased planktonic cells. Moreover, electrochemical tests using AQ injection (100 ppm in the medium) at 3-d incubation further endorsed the superior performance of AQ based on 120% polarization resistance (R_p) increase compared to 84% achieved by 100 ppm THPS injection.

Key words: *Desulfovibrio ferrophilus* IS5 biofilm, carbon steel, anthraquinone, microbiologically influenced corrosion

INTRODUCTION

Biofilms, as defined by the International Union of Pure and Applied Chemistry (IUPAC), are aggregates of microorganisms in which cells adhere to each other and/or to a surface, and are embedded within a self-secreted extracellular polymeric substance (EPS) [1]. EPS contains polysaccharides, proteins, and extracellular DNA. Biofilms have their own ecosystems that are different from the surrounding environments. Their presence and localization determine their impact, which can be both clinical and environmental. In clinical settings, biofilms are a major cause of persistent infections [2].

Biofilms exhibit various defense mechanisms, including diffusional barriers to slow down biocide penetration, lowered metabolic rate to minimize uptake of a biocide, formation of tough persister cells to preserve the biofilm for rapid future recovery, and upregulation of genes for enzymes that degrade biocide molecules. Sessile cells can also utilize efflux pumps to pump toxic chemicals out of the cells [3–5]. Bacteria within biofilms can exhibit up to 1,000-fold greater resistance to antibiotics and antimicrobial agents than planktonic cells [6], contributing to the emergence of antibiotic-resistant strains of infectious bacteria [7], which represents a critical and pressing public health crisis. Electroactive biofilms such as some sulfate reducing bacteria (SRB) and nitrate reducing bacteria (NRB) biofilms (e.g., anaerobic *Pseudomonas aeruginosa* biofilm) can harvest electrons directly from energetic metals such as carbon steel to produce energy for metabolism [8]. Both corrosive metabolites and direct electron transport can lead to microbiologically influenced corrosion (MIC), also known as biocorrosion [9]. Biofilms are very corrosive since sessile cells are capable of direct electron transport at the surface of the metal and can concentrate corrosive metabolites underneath the biofilms [10,11]. In addition to causing MIC, biofilms cause biofouling, which can reduce flow rates, or heat transfer efficiency in heat exchangers, and equipment failures [12]. Biofilms in potable water pipelines pose contamination risks, leading to waterborne diseases and chemical exposure [13,14].

MIC is a threat to pipeline infrastructure, particularly in the oil, gas, and water utilities sectors, accounting for 20-40% of severe corrosion incidents and more than US\$2.7 trillion in annual costs globally [2,9,15]. A variety of strategies, particularly those involving physical and chemical approaches, have been implemented to combat the formation of corrosive biofilms and mitigate MIC in industrial settings [9]. One of the primary industries affected by SRB-induced corrosion is the oil and gas sector, where pipelines are especially vulnerable [2]. *Desulfovibrio ferrophilus* IS5, a highly corrosive SRB, was shown to cause 3.3 mm/year uniform corrosion on X65 carbon steel (CS) over 30-d in enriched artificial seawater (EASW) at 28 °C [16]. Pipeline biofilms are commonly controlled by pigging, which mechanically scrapes internal surfaces, and by biocide treatments (e.g., chlorine, glutaraldehyde) which kill microorganisms [9,17], however, some biocides can be toxic and environmentally problematic. Alternative strategies include specialized iron alloys that release biocidal metal ions (copper, zinc, aluminum, lead) to inhibit SRB activity, biofilm formation, and corrosion [18]. In addition to mechanical and chemical methods, antibacterial coatings and biobased agents provide promising strategies to limit biofilm formation [19–21]. These coatings prevent microbial attachment, while natural compounds such as D-amino acids [22], plant-derived antimicrobials like D-limonene, and biosurfactants suppress biofilm development and microbial activity, offering environmentally friendly corrosion control [23].

Given the environmental risks associated with traditional chemical treatments, there is growing interest in alternative, eco-friendly antibacterial agents to control SRB biofilms in field applications. This study explored the potential of unsubstituted 9,10-anthraquinone (AQ), a plant-based antibacterial agent [24] for combating biofilm formation and MIC by SRB. AQ is a polycyclic quinone compound containing a cyclohexadiene diketone structure [24]. It suppresses SRB growth by targeting hydrogen-dependent sulfate respiration [25], likely acting as a redox uncoupler that disrupts electron transfer and ATP synthesis. Its interaction with cytochrome c3 suggests interference with the electron transport chain, reducing H₂S production. Previous studies have reported direct electron transport between other bacterial species and iron which involved the use of outer membrane, c3-type, cytochromes [26,27].

AQ and its derivatives occur naturally in a variety of sources, including plants [28] and fungi [29]. These compounds have long been used in traditional Chinese medicine, valued for their diverse biological activities, including anti-inflammatory, anticancer, and antibacterial effects [24]. Rhein, an anthraquinone, exhibited antibacterial activity against several species, including *Staphylococcus*, *Streptococcus*, and *Bacillus*. Its inhibitory mechanism involves blocking electron transfer in the respiratory chain, which disrupts nucleic acid and protein synthesis, thereby severely limiting bacterial growth. [24,30,31]. In *Streptococcus mutans*, rhein disrupted cell wall integrity, inhibited biofilm formation, glycolysis, and altered bacterial morphology exerting a bacteriostatic effect [24,32].

EXPERIMENTAL PROCEDURE

2. Materials and methods

2.1 Bacterium, culture medium, and metal

D. ferrophilus IS5, a pure-strain SRB known for its high corrosivity, was cultivated in EASW culture medium at an optimal growth temperature of 28 °C, as outlined in the test matrix presented in Table 1. Table 2 shows the composition of the EASW medium. The initial pH of the culture medium was adjusted to 7.0 by using a 5% (w/w) NaOH solution. After autoclaving the medium for sterilization, it was sparged using a gas distributor with filter-sterilized nitrogen (N₂) gas for one hour to eliminate dissolved oxygen. The medium was then transferred to an anaerobic chamber, where filter-sterilized L-cysteine (100 ppm), acting as an oxygen scavenger, and a filter-sterilized Fe²⁺ solution (20 ppm), serving as an enzyme cofactor to enhance SRB growth [33], were added.

Table 1
Test matrix for AQ efficacy testing

Parameter	Condition
Microbe	<i>D. ferrophilus</i> IS5 (DSM 15579)
Cultural medium	EASW
Coupon material	X65 with working surface area of 1 cm ² . Each coupon was polished to 600 grit.
Anaerobic vials	Six 125 mL vials each containing 3 coupons in 50 mL EASW medium and 0.5 mL <i>D. ferrophilus</i> inoculum. The first vial served as the control (without AQ), while the remaining 5 vials contained 5, 10, 15, 20, and 25 ppm AQ, respectively.

Electrochemical glass cells	10 mL glass cells with each containing a single 1 cm ² working electrode (WE) coated with epoxy coating on all sides except the exposed working surface area, graphite counter/pseudo-reference electrode (CE/p-RE) submerged in 5 mL EASW inoculated with 0.05 mL seed culture.
Initial pH	7.0 ± 0.2
Initial cell count	6.73 (± 0.80) × 10 ⁵ cells/mL right after inoculation.
Incubation time	7 d
Assay	Sessile cell count, planktonic cell count, weight loss, biofilm visualization. Electrochemical tests: LPR (before and after tandem injections of 100 ppm AQ and THPS).

Table 2
Composition of EASW culture medium [34]

Chemical	Amount
NaCl	23.476 g
Na ₂ SO ₄	3.917 g
NaHCO ₃	0.192 g
KCl	0.664 g
KBr	0.096 g
H ₃ BO ₃	0.026 g
MgCl ₂	4.965 g
SrCl ₂ ·6H ₂ O	0.04 g
CaCl ₂ ·2H ₂ O	1.469 g
Sodium lactate	3.5 g
Yeast extract	1.0 g
Sodium citrate	0.5 g
MgSO ₄ ·7H ₂ O	0.71 g
CaSO ₄ ·2H ₂ O	0.1 g
NH ₄ Cl	0.1 g
K ₂ HPO ₄	0.05 g
Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	1.37 g
Deionized water	1 L

X65 coupons each with a top exposed surface area of 1 cm² were used in this experiment. The remaining surfaces of these coupons were coated with an inert epoxy coating (3M Product 323). Only the top surfaces of the coupons were abraded to a 600 grit finish. Table 3 shows the elemental composition of X65. All coupons were thoroughly cleaned using pure isopropanol and subsequently sanitized under UV light for several hours. At inoculation, AQ suspension (with a mean particle size of 2 µm) provided by EB Technologies (Dallas, Texas)¹, and THPS solution

¹ EB Technologies, Inc., Dallas, TX.

(Product Number: 15175, Sigma-Aldrich®)² were added to their respective anaerobic vials according to the defined concentrations.

Table 3
Elemental composition (wt. %) of X65 [35]

Metal	C	Si	Mn	P	S	V	Nb	Ti	Fe
X65	0.16	0.45	1.65	0.02	0.01	0.09	0.05	0.06	Balance

2.2 Enumeration of sessile cells

After 7-d incubation at 28 °C, all coupons were retrieved and washed with a pH 7.4 phosphate-buffered saline (PBS) solution. The coupon replicates for sessile cell counts were processed by removing the biofilm using sterilized cell scrapers and re-suspending the biomass in 10 mL of PBS solution. Both sessile cell suspensions and planktonic cell suspensions obtained from the same anaerobic vials were then vortexed to evenly distribute the cells in the liquid samples before being visualized under a microscope at 400X magnification for cell counting using a hemocytometer [16]. Because *D. ferrophilus* cells were motile, they were easily distinguished from non-cell particles such as FeS particles under a 400X microscope.

2.3 Confocal laser scanning microscopy (CLSM) analysis

Biofilms on the respective replicate coupons were stained with the Live/Dead® BacLight™ Bacterial Viability Kit L7012 (Life Technologies, Grand Island, NY, USA)³, which employed SYTO 9 as a green-fluorescent stain for live cells and propidium iodide as a red-fluorescent stain for dead cells [36]. In the resulting CLSM images, live cells appeared green, while dead cells were represented in red.

2.4 Weight loss analysis

The coupon replicates for weight loss measurements were washed with a fresh Clarke's solution to remove adherent corrosion products according to the standard protocol (ASTM G1–03). After treatment with the Clarke's solution, the coupons were weighed to determine the changes in weight before and after incubation with SRB. For each weight loss data point, an average of three replicate coupons from the same anaerobic bottle were used.

2.5 Electrochemical tests

A PCI4/750 potentiostat (Gamry Instruments, Inc., Warminster, PA, USA) was utilized to evaluate the electrochemical responses of X65 coupon working electrodes (WEs) with graphite as counter electrode/pseudo reference electrode (CE/p-RE) in a 2-electrode configuration before and after tandem injections of 100 ppm AQ and 100 ppm THPS, respectively in 10 mL biofilm test kit (mini glass cells) [37] with 5 mL culture medium inoculated with *D. ferrophilus* at 3 d. Linear polarization resistance (LPR) scans were conducted at a rate of 0.1667 mV/s over a range from –10 mV to +10 mV relative to the open circuit potential (OCP) [37].

² SIGMA-ALDRICH® is a registered trademark of Merck KGaA, Darmstadt, Germany.

³ Live/Dead® and BacLight™ are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

RESULTS AND DISCUSSION

3.1 Sessile cell count and weight loss data

In this work, AQ caused substantial reductions in sessile cell counts and corrosion rates without significantly affecting planktonic SRB populations, likely because the particle-like behavior of AQ specifically targeted biofilm formation rather than free-floating bacteria. The black broth color intensities in Figure 1A appear similar for all AQ concentrations, indicating no impact of AQ on planktonic growth. This is validated by the planktonic cell count data in Table 4. Preliminary results using *D. ferrophilus* IS5 indicated that AQ, at 15 ppm and above (up to 25 ppm), reduced sessile cell counts by over 80% and significantly decreases metal weight loss due to corrosion (Table 4). Figure 1B clearly shows a higher AQ dosage led to cleaner coupon surface after incubation. After 7-d of incubation, sessile cell counts demonstrated a strong dose-dependent inhibition of *D. ferrophilus* IS5 biofilm by AQ, which was added at the inoculation stage (0 d) to prevent biofilm formation as shown in Figure 1B and Table 4. At the highest concentration tested (25 ppm), sessile cell counts remained below the hemocytometer detection limit ($< (2.3 \pm 1.2) \times 10^4$ cells/cm²), calculated from the biofilm resuspension volume per cm² coupon surface and the cells suspension detection limit of 5×10^4 cells/mL for the hemocytometer), indicating highly effective and consistent inhibition, with a $> 99.9\%$ (> 3 -log) reduction in sessile cell count and a 99.7% reduction in weight loss compared to the untreated control (no AQ).

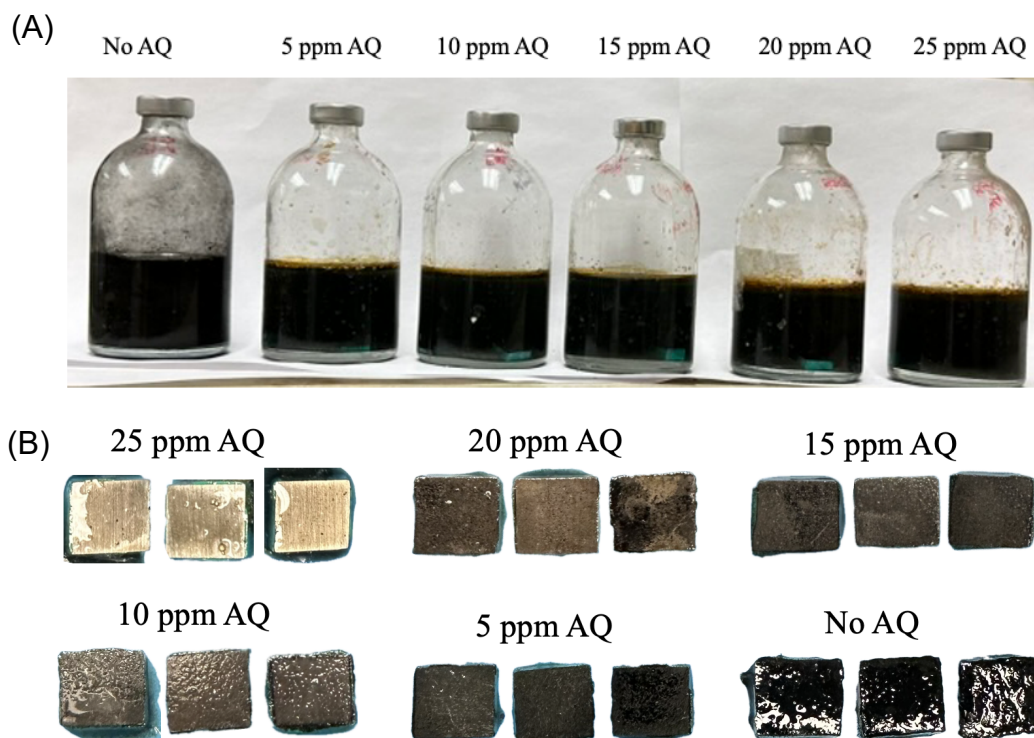


Figure 1. (A) 125 mL anaerobic vials with 50 mL EASW containing X65 coupons with and without AQ after 7-d *D. ferrophilus* IS5 incubation, (B) coupons after the incubation.

Table 4
Summary of average cell counts and weight loss data of X65 coupons after 7-d of
***D. ferrophilus* IS5 incubation with and without AQ. (n=3, $\pm$ s.d)**

AQ	Sessile cell count (10^6 cells/cm ²)	Planktonic cell count (10^6 cells/mL)	Weight loss (mg/cm ²)
0 ppm	38.8 ($\pm$ 2.94)	3.73 ($\pm$ 0.15)	12.7 $\pm$ 2.3
5 ppm	11.4 ($\pm$ 0.65) (71% decrease)	3.66 ($\pm$ 0.18)	3.80 $\pm$ 0.95 (70% decrease)
10 ppm	9.85 ($\pm$ 0.51) (75% decrease)	3.56 ($\pm$ 0.15)	2.87 $\pm$ 0.99 (77% decrease)
15 ppm	7.90 ($\pm$ 0.26) (80% decrease)	3.52 ($\pm$ 0.15)	1.13 $\pm$ 0.45 (91% decrease)
20 ppm	3.17 ($\pm$ 0.40) (92% decrease)	3.47 ($\pm$ 0.14)	0.23 $\pm$ 0.15 (98% decrease)
25 ppm	Undetectable ($< (2.3 \pm 1.2) \times 10^4$ cells/cm ²) ($> 99.9\%$ or > 3 -log decrease)	3.41 ($\pm$ 0.20)	0.03 $\pm$ 0.06 (99.7% decrease)

At 25 ppm, THPS significantly inhibited *D. ferrophilus* biofilm formation on X65 coupons (Figure 2B), with sessile cell counts falling below the hemocytometer detection limit ($1.67 (\pm 0.58) \times 10^4$ cells/cm²) calculated based on sessile cell re-suspension dilution and 5×10^4 cells/mL detection limit for the cell suspension), indicating highly effective and consistent biofilm prevention. Unlike AQ, THPS also markedly reduced planktonic cell counts, which were similarly below the detection limit (Figure 2A). Weight loss data reflected THPS efficacy (Table 5), with coupons exposed to 25 ppm THPS showing a weight loss of 0.07 ± 0.06 mg/cm² (99.4% decrease), compared to 0.03 ± 0.06 mg/cm² (99.7% decrease) under 25 ppm AQ, confirming that both treatments very effectively mitigated corrosion, though THPS had a slightly higher weight loss than AQ.

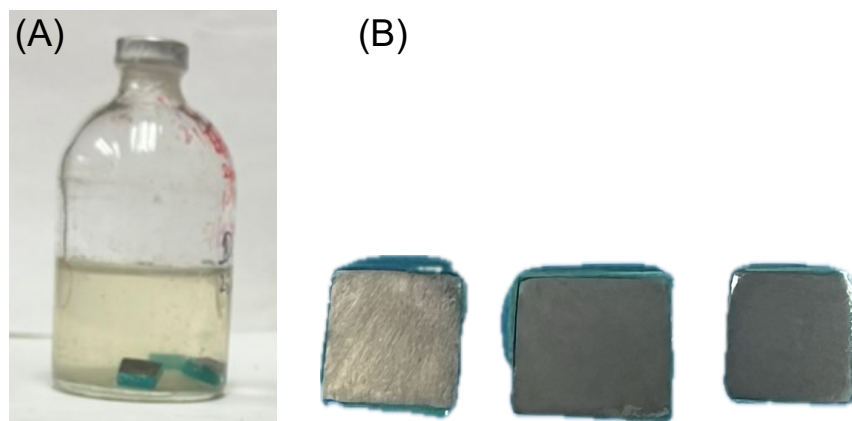


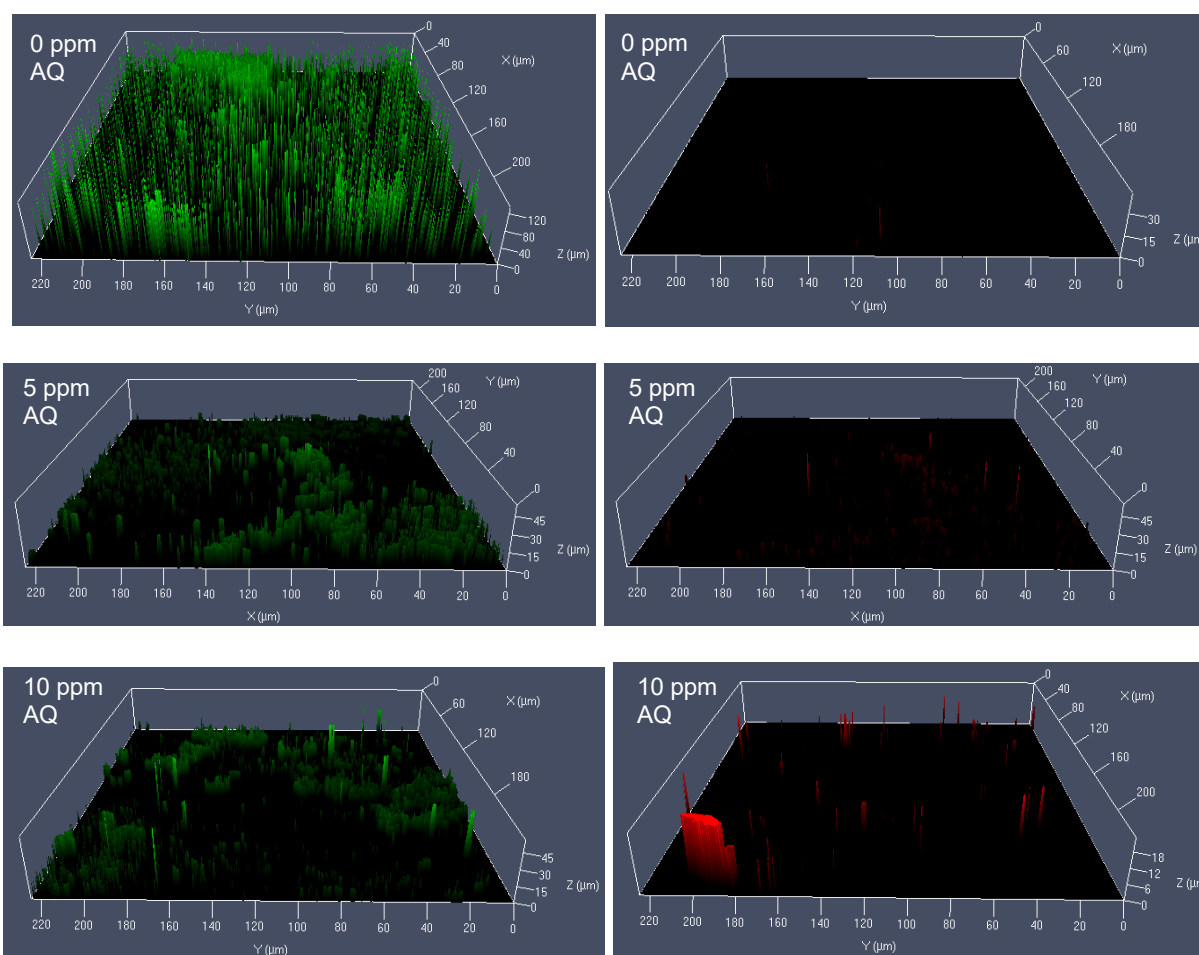
Figure 2. (A) 125 mL anaerobic vial with 50 mL EASW containing X65 coupons with 25 ppm THPS after 7-d *D. ferrophilus* IS5 incubation, (B) coupons after the incubation.

Table 5
Summary of cell counts and average weight loss data of X65 coupons after 7-d of
***D. ferrophilus* IS5 incubation with 25 ppm THPS. (n=3, $\pm$ s.d.)**

THPS	Sessile cell count (10^6 cells/cm ²)	Planktonic cell count (10^6 cells/mL)	Weight loss (mg/cm ²)
25 ppm	Undetectable ($< (2.3 \pm 1.2) \times 10^4$ cells/cm ²) ($> 99.9\%$ or > 3 -log decrease)	Undetectable (< 5 $\times 10^4$ cells/mL) ($> 99.2\%$ decrease)	0.07 ± 0.06 (99.4% decrease)

3.2 CLSM analysis

CLSM 3D image analysis of *D. ferrophilus* biofilms on X65 coupons at 7 d revealed a clear dose-dependent inhibition of biofilm formation in response to AQ treatment as shown in Figure 3. Untreated coupons (0 ppm AQ) exhibited dense and thick biofilm, whereas progressive reductions in biofilm biomass and coverage were observed at 5, 10, 15, and 20 ppm AQ, with the highest level of prevention observed at 25 ppm AQ. Notably, treatment with 25 ppm THPS (Figure 4) produced a comparable level of biofilm inhibition, indicating that both compounds are effective in mitigating SRB biofilm formation on steel surfaces.



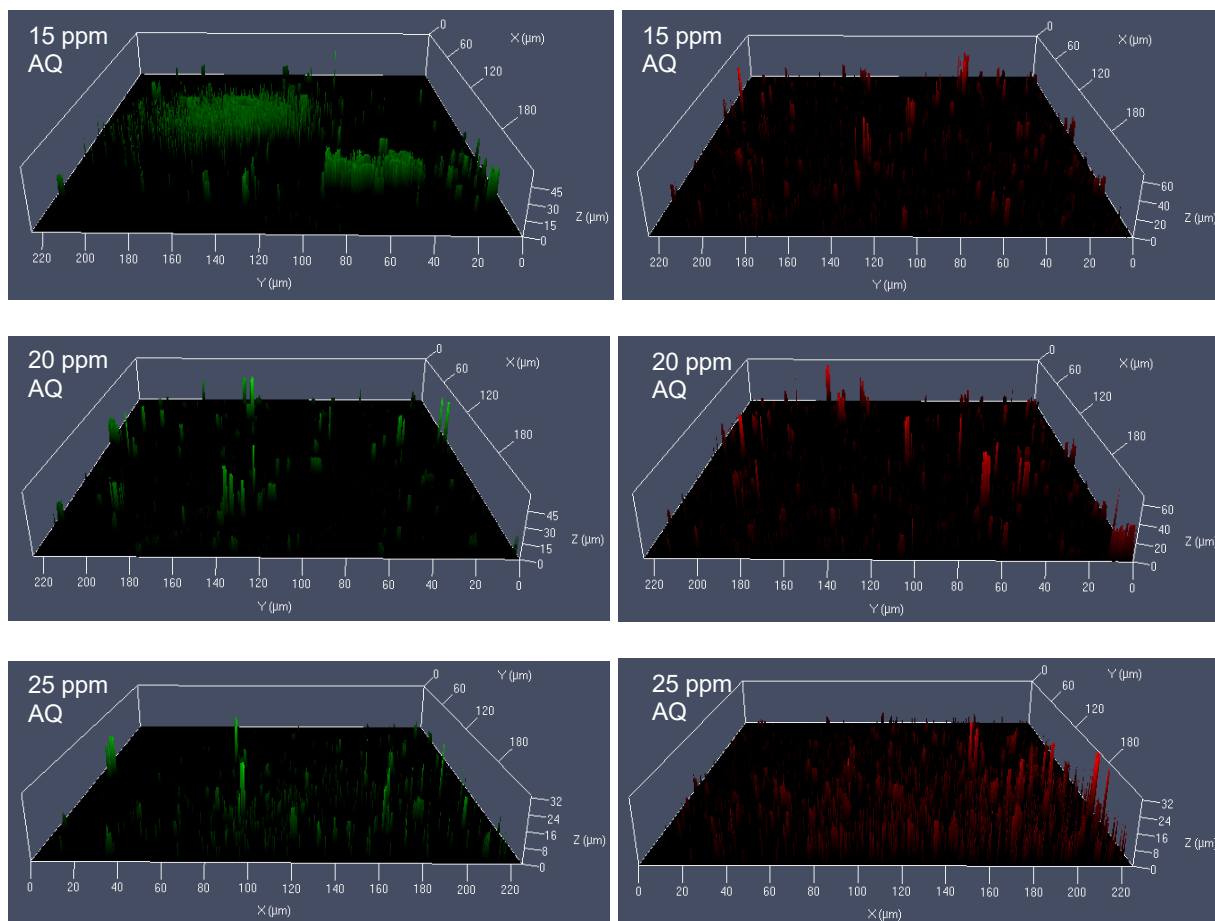


Figure 3. CLSM 3D images of *D. ferrophilus* IS5 biofilm on X65 coupons at different concentrations of AQ at 7 d of anaerobic incubation. The green columns represent live cells (left panel), and the red columns represent dead cells (right panel), showing a dose-dependent reduction in biofilm formation, with the highest inhibition observed at 25 ppm AQ.

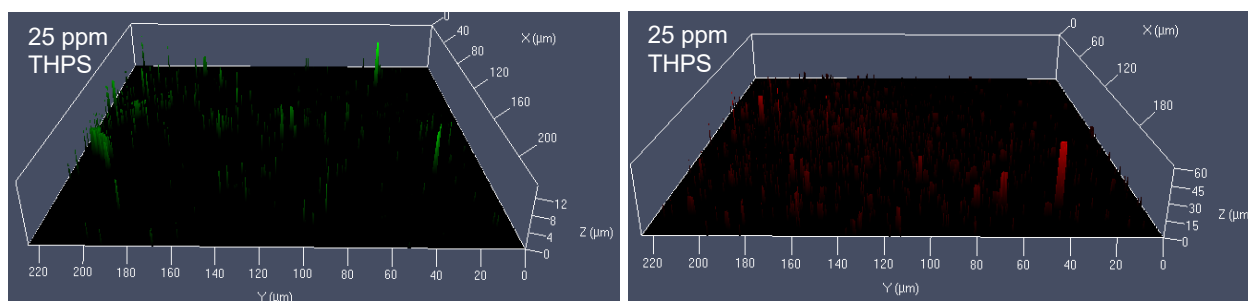


Figure 4. CLSM 3D images of *D. ferrophilus* IS5 biofilm on X65 coupons at 25 ppm THPS at 7 d of anaerobic incubation. The green columns represent live cells (left panel), and the red columns represent dead cells (right panel). Treatment with 25 ppm THPS inhibited biofilm formation to a level comparable to 25 ppm AQ.

3.3 Electrochemical tests

LPR scans at 3 d of incubation were conducted (with intervals) before and after tandem injections of AQ (100 ppm in the medium) and THPS (100 ppm in the medium) in their respective biofilm test kit vials as shown in Figure 5.

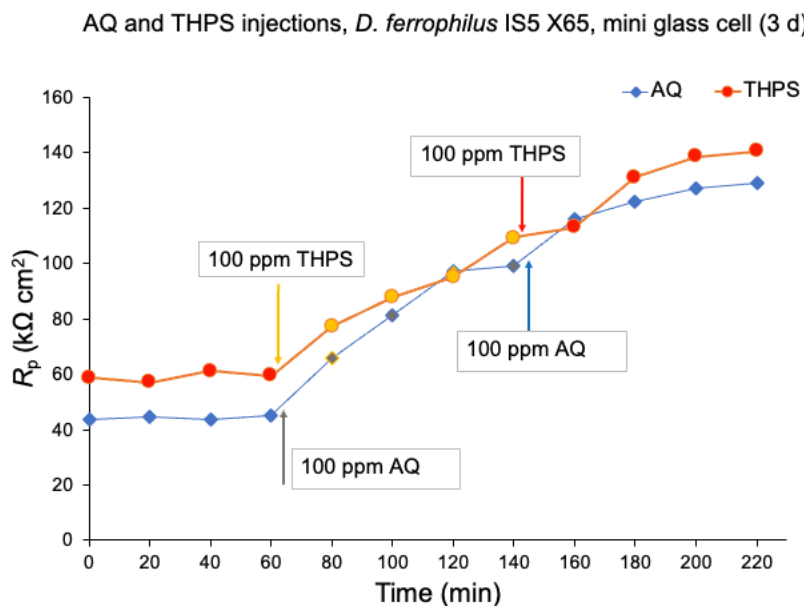


Figure 5. R_p values for X65 single WEs (without any replicates) before and after tandem injections of 100 ppm AQ and 100 ppm THPS at 3 d of *D. ferrophilus* IS5 incubation.

Upon the first 100 ppm AQ injection, AQ increased the polarization resistance (R_p) by 120%, outperforming THPS, which resulted in an 84% increase. This suggests that AQ exerted a rapid and potent inhibitory effect on corrosion processes. A second 100 ppm injection produced smaller but consistent increases in R_p , 30% for AQ and 28% for THPS. While THPS is an established biocide, this work shows that AQ demonstrated similar efficacy as THPS against *D. ferrophilus* IS5, highlighting its potential as a corrosion inhibitor against SRB MIC.

CONCLUSIONS

- 25 ppm AQ strongly inhibited *D. ferrophilus* IS5 biofilm formation over a 7-d period when applied at inoculation.
- Sessile cell count reduced > 99.9% at 25 ppm AQ, but planktonic cell count was reduced by only 9%, indicating selective inhibition of sessile cells.
- Weight loss-based corrosion rate decreased 99.7% on average by 25 ppm AQ which was similar to 25 ppm THPS.
- AQ and THPS performed similarly at the same concentration (100 ppm) against a mature *D. ferrophilus* IS5 biofilm with a 120% increase compared to an 84% increase in R_p , respectively.

REFERENCES

- [1] M. Vert, Y. Doi, K.-H. Hellwich, M. Hess, P. Hodge, P. Kubisa, M. Rinaudo, F. Schué, Terminology for biorelated polymers and applications (IUPAC Recommendations 2012), *Pure and Applied Chemistry* 84 (2012) 377–410. <https://doi.org/10.1351/PAC-REC-10-12-04>.
- [2] M. Cámara, W. Green, C.E. MacPhee, P.D. Rakowska, R. Raval, M.C. Richardson, J. Slater-Jefferies, K. Steventon, J.S. Webb, Economic significance of biofilms: a multidisciplinary and cross-sectoral challenge, *Npj Biofilms and Microbiomes* 8 (2022) 42.
- [3] S.M. Soto, Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm, *Virulence* 4 (2013) 223–229. <https://doi.org/10.4161/viru.23724>.
- [4] I. Alav, J.M. Sutton, K.M. Rahman, Role of bacterial efflux pumps in biofilm formation, *Journal of Antimicrobial Chemotherapy* 73 (2018) 2003–2020. <https://doi.org/10.1093/jac/dky042>.
- [5] H.V. Acker, T. Coenye, The Role of Efflux and Physiological Adaptation in Biofilm Tolerance and Resistance *, *Journal of Biological Chemistry* 291 (2016) 12565–12572. <https://doi.org/10.1074/jbc.R115.707257>.
- [6] H. Ceri, M.E. Olson, C. Stremick, R.R. Read, D. Morck, A. Buret, The Calgary Biofilm Device: New Technology for Rapid Determination of Antibiotic Susceptibilities of Bacterial Biofilms, *Journal of Clinical Microbiology* 37 (1999) 1771–1776. <https://doi.org/10.1128/jcm.37.6.1771-1776.1999>.
- [7] V. Cepas, Y. López, E. Muñoz, D. Rolo, C. Ardanuy, S. Martí, M. Xercavins, J.P. Horcajada, J. Bosch, S.M. Soto, Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria, *Microbial Drug Resistance* 25 (2019) 72–79. <https://doi.org/10.1089/mdr.2018.0027>.
- [8] Y. Li, D. Xu, C. Chen, X. Li, R. Jia, D. Zhang, W. Sand, F. Wang, T. Gu, Anaerobic microbiologically influenced corrosion mechanisms interpreted using bioenergetics and bioelectrochemistry: a review, *Journal of Materials Science & Technology* 34 (2018) 1713–1718.
- [9] D. Xu, T. Gu, D.R. Lovley, Microbially mediated metal corrosion, *Nature Reviews Microbiology* 21 (2023) 705–718. <https://doi.org/10.1038/s41579-023-00920-3>.
- [10] Extracellular electron transfer mechanisms between microorganisms and minerals | *Nature Reviews Microbiology*, (n.d.). <https://www.nature.com/articles/nrmicro.2016.93> (accessed September 28, 2025).
- [11] J. Zhao, F. Li, Y. Cao, X. Zhang, T. Chen, H. Song, Z. Wang, Microbial extracellular electron transfer and strategies for engineering electroactive microorganisms, *Biotechnology Advances* 53 (2021) 107682. <https://doi.org/10.1016/j.biotechadv.2020.107682>.
- [12] Biofouling and Mitigation Methods: A Review, *Biointerface Res Appl Chem* 13 (2022) 185. <https://doi.org/10.33263/BRIAC132.185>.
- [13] N.R. Council, D. on E. and L. Studies, W.S. and T. Board, C. on P.W.S.D.S.A. and R. Risks, *Drinking Water Distribution Systems: Assessing and Reducing Risks*, National Academies Press, 2007.
- [14] P.R. Hunter, J.M. Colford, M.W. LeChevallier, S. Binder, P.S. Berger, Waterborne diseases., *Emerg Infect Dis* 7 (2001) 544.
- [15] F.S. Marquis, *Proceedings of the 8th Pacific Rim International Conference on Advanced Materials and Processing (PRICM-8)*, Springer, 2017.
- [16] A. Khan, L. Xu, P. Kijla, S. Kumseranee, S. Punpruk, T. Gu, Impact of gravity on biofilm growth and corrosion of X65 pipeline steel by a sulfate reducing bacterium, *Corrosion Science* 234 (2024) 112127. <https://doi.org/10.1016/j.corsci.2024.112127>.

- [17] C. Cote, O. Rosas, M. Szttyler, J. Doma, I. Beech, R. Basseguy, Corrosion of low carbon steel by microorganisms from the 'pigging' operation debris in water injection pipelines, *Bioelectrochemistry* 97 (2014) 97–109. <https://doi.org/10.1016/j.bioelechem.2013.11.001>.
- [18] H.H.P. Fang, L.-C. Xu, K.-Y. Chan, Effects of toxic metals and chemicals on biofilm and biocorrosion, *Water Research* 36 (2002) 4709–4716. [https://doi.org/10.1016/S0043-1354\(02\)00207-5](https://doi.org/10.1016/S0043-1354(02)00207-5).
- [19] M. Villegas, C. Alonso-Cantu, S. Rahmani, D. Wilson, Z. Hosseinidoust, T.F. Didar, Antibiotic-Impregnated Liquid-Infused Coatings Suppress the Formation of Methicillin-Resistant *Staphylococcus aureus* Biofilms, *ACS Appl. Mater. Interfaces* 13 (2021) 27774–27783. <https://doi.org/10.1021/acsami.0c19355>.
- [20] A. Majeed, F. Sagar, A. Latif, H. Hassan, A. Iftikhar, R.O. Darouiche, M.A. Mohajer, Does antimicrobial coating and impregnation of urinary catheters prevent catheter-associated urinary tract infection? A review of clinical and preclinical studies, *Expert Review of Medical Devices* 16 (2019) 809–820. <https://doi.org/10.1080/17434440.2019.1661774>.
- [21] W.M. Abd El-Gawad, N.M. Ahmed, W.S. Mohamed, E.R. Souaya, A novel approach for new cost-saving durable anticorrosive and antibacterial coatings, *J Coat Technol Res* 16 (2019) 1263–1281. <https://doi.org/10.1007/s11998-019-00202-9>.
- [22] Y. Li, R. Jia, H.H. Al-Mahamedh, D. Xu, T. Gu, Enhanced Biocide Mitigation of Field Biofilm Consortia by a Mixture of D-Amino Acids, *Front. Microbiol.* 7 (2016). <https://doi.org/10.3389/fmicb.2016.00896>.
- [23] Y. Lou, W. Chang, T. Cui, J. Wang, H. Qian, L. Ma, X. Hao, D. Zhang, Microbiologically influenced corrosion inhibition mechanisms in corrosion protection: A review, *Bioelectrochemistry* 141 (2021) 107883. <https://doi.org/10.1016/j.bioelechem.2021.107883>.
- [24] T. Qun, T. Zhou, J. Hao, C. Wang, K. Zhang, J. Xu, X. Wang, W. Zhou, Antibacterial activities of anthraquinones: structure–activity relationships and action mechanisms, *RSC Med Chem* 14 (n.d.) 1446–1471. <https://doi.org/10.1039/d3md00116d>.
- [25] F.I. Cooling, C.L. Maloney, E. Nagel, J. Tabinowski, J.M. Odom, Inhibition of sulfate respiration by 1,8-dihydroxyanthraquinone and other anthraquinone derivatives, *Applied and Environmental Microbiology* 62 (1996) 2999–3004. <https://doi.org/10.1128/aem.62.8.2999-3004.1996>.
- [26] H.-Y. Tang, D.E. Holmes, T. Ueki, P.A. Palacios, D.R. Lovley, Iron Corrosion via Direct Metal-Microbe Electron Transfer, *mBio* 10 (2019) 10.1128/mbio.00303-19. <https://doi.org/10.1128/mbio.00303-19>.
- [27] H.-Y. Tang, C. Yang, T. Ueki, C.C. Pittman, D. Xu, T.L. Woodard, D.E. Holmes, T. Gu, F. Wang, D.R. Lovley, Stainless steel corrosion via direct iron-to-microbe electron transfer by *Geobacter* species, *The ISME Journal* 15 (2021) 3084–3093. <https://doi.org/10.1038/s41396-021-00990-2>.
- [28] J. Duval, V. Pecher, M. Poujol, E. Lesellier, Research advances for the extraction, analysis and uses of anthraquinones: A review, *Industrial Crops and Products* 94 (2016) 812–833. <https://doi.org/10.1016/j.indcrop.2016.09.056>.
- [29] H. Huang, F. Wang, M. Luo, Y. Chen, Y. Song, W. Zhang, S. Zhang, J. Ju, Halogenated Anthraquinones from the Marine-Derived Fungus *Aspergillus* sp. SCSIO F063, *J. Nat. Prod.* 75 (2012) 1346–1352. <https://doi.org/10.1021/np3002699>.
- [30] Y.-X. Zhou, W. Xia, W. Yue, C. Peng, K. Rahman, H. Zhang, Rhein: A Review of Pharmacological Activities, *Evidence-Based Complementary and Alternative Medicine* 2015 (2015) 578107. <https://doi.org/10.1155/2015/578107>.

- [31] L. Yu, H. Xiang, J. Fan, D. Wang, F. Yang, N. Guo, Q. Jin, X. Deng, Global transcriptional response of *Staphylococcus aureus* to Rhein, a Natural Plant Product, *Journal of Biotechnology* 135 (2008) 304–308. <https://doi.org/10.1016/j.jbiotec.2008.04.010>.
- [32] V. Folliero, F. Dell'Annunziata, E. Roscetto, A. Amato, R. Gasparro, C. Zannella, V. Casolaro, A. De Filippis, M.R. Catania, G. Franci, M. Galdiero, Rhein: A novel antibacterial compound against *Streptococcus mutans* infection, *Microbiological Research* 261 (2022) 127062. <https://doi.org/10.1016/j.micres.2022.127062>.
- [33] R. Jia, D. Wang, P. Jin, T. Unsal, D. Yang, J. Yang, D. Xu, T. Gu, Effects of ferrous ion concentration on microbiologically influenced corrosion of carbon steel by sulfate reducing bacterium *Desulfovibrio vulgaris*, *Corrosion Science* 153 (2019) 127–137. <https://doi.org/10.1016/j.corsci.2019.03.038>.
- [34] D. Wang, P. Kijkla, M.E. Mohamed, M.A. Saleh, S. Kumseranee, S. Punpruk, T. Gu, Aggressive corrosion of carbon steel by *Desulfovibrio ferrophilus* IS5 biofilm was further accelerated by riboflavin, *Bioelectrochemistry* 142 (2021) 107920.
- [35] D. Wang, J. Liu, R. Jia, W. Dou, S. Kumseranee, S. Punpruk, X. Li, T. Gu, Distinguishing two different microbiologically influenced corrosion (MIC) mechanisms using an electron mediator and hydrogen evolution detection, *Corrosion Science* 177 (2020) 108993.
- [36] E. Zhou, Y. Yang, J. Li, G. Ren, L. Li, Z. Li, Y. Lu, T. Gu, F. Wang, D. Xu, A Novel Cu and Ag Bearing High-Entropy Alloy With Remarkable Antibacterial, Antiviral and Anti-Microbial Corrosion Performances, *Advanced Functional Materials* 35 (2025) 2419467. <https://doi.org/10.1002/adfm.202419467>.
- [37] L. Xu, A. Khan, S.A. Aqeel, A. Alqahtani, L. AlSharif, P. Kijkla, S. Kumseranee, S. Punpruk, T. Gu, Distinguishing abiotic corrosion from two types of microbiologically influenced corrosion (MIC) using a new electrochemical biofilm/MIC test kit, *Journal of Environmental Management* 374 (2025) 124093. <https://doi.org/10.1016/j.jenvman.2025.124093>.