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Mitigation of carbon steel biocorrosion using a green biocide enhanced by a nature-mimicking anti-biofilm peptide in a flow loop

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Abstract

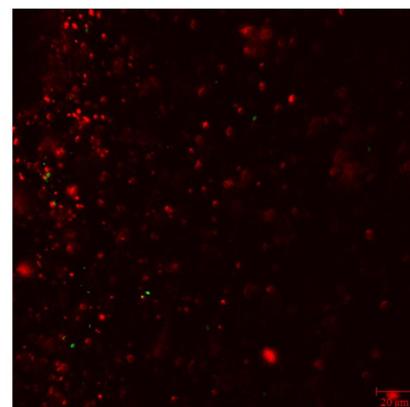
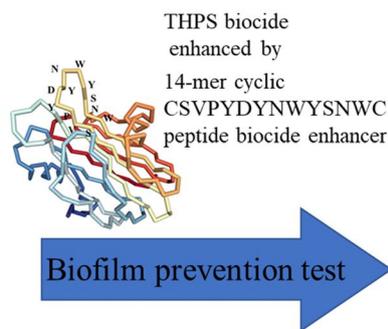
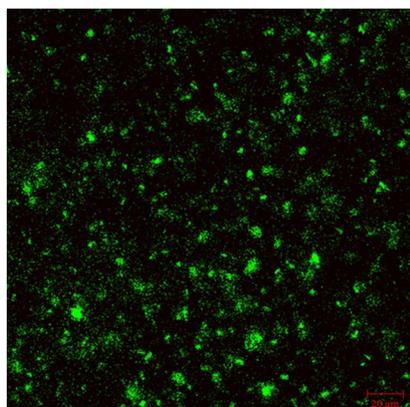
Biocorrosion, also called microbiologically influenced corrosion (MIC), is a common operational threat to many industrial processes. It threatens carbon steel, stainless steel and many other metals. In the bioprocessing industry, reactor vessels in biomass processing and bioleaching are prone to MIC. MIC is caused by biofilms. The formation and morphology of biofilms can be impacted by fluid flow. Fluid velocity affects biocide distribution and MIC. Thus, assessing the efficacy of a biocide for the mitigation of MIC under flow condition is desired before a field trial. In this work, a benchtop closed flow loop bioreactor design was used to investigate the biocide mitigation of MIC of C1018 carbon steel at 25 °C for 7 days using enriched artificial seawater. An oilfield biofilm consortium was analyzed using metagenomics. The biofilm consortium was grown anaerobically in the flow loop which had a holding vessel for the culture medium and a chamber to hold C1018 carbon steel coupons. Peptide A (codename) was a chemically synthesized cyclic 14-mer (cys-ser-val-pro-tyr-asp-tyr-asn-trp-tyr-ser-asn-trp-cys) with its core 12-mer sequence originated from a biofilm dispersing protein secreted by a sea anemone which possesses a biofilm-free exterior. It was used as a biocide enhancer. The combination of 50 ppm (w/w) THPS (tetrakis hydroxymethyl phosphonium sulfate) biocide + 100 nM (180 ppb by mass) Peptide A resulted in extra 1-log reduction in the sulfate reducing bacteria (SRB) sessile cell count and the acid producing bacteria (APB) sessile cell count compared to 50 ppm THPS alone treatment. Furthermore, with the enhancement of 100 nM Peptide A, extra 44% reduction in weight loss and 36% abatement in corrosion pit depth were achieved compared to 50 ppm THPS alone treatment.

Keywords: Microbiologically influenced corrosion (MIC), Biofilm, Flow loop, Biocide, Biocide enhancer, Peptide

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Graphical Abstract



Introduction

MIC (microbiologically influenced corrosion) is a common threat to many industrial processes and settings, such as oil and gas operations (Rasheed et al. 2019), storage tanks (Parthipan et al. 2018), reactors including those used to process biomass and those used to bioleach ores (Chang et al. 2014; Kumar and Sharma 2017; Dong et al. 2018; Kaliyaraj et al. 2019; Wu et al. 2021; Bimestre et al. 2022), biomedical implants (Kabir et al. 2021), etc. It reduces the service life of oil and gas pipelines and can potentially lead to catastrophes (Gieg et al. 2011; Qian et al. 2018; Wei et al. 2021). Microbes blamed for pipeline MIC include sulfate reducing bacteria (SRB), acid producing bacteria (APB), acetogenic bacteria and methanogens (Sherar et al. 2011; Park et al. 2011). In field operations, SRB, APB and other microorganisms usually co-exist in a biofilm consortium (Wang et al. 2020b). SRB biofilms are often the main culprit of MIC due to their prevalence in the field environments, such as oil and gas pipelines which are usually kept oxygen free. They can utilize sulfate as the terminal electron acceptor for the oxidation of a carbon source or H_2 as the electron donor (Gu et al. 2021; Vigneron et al. 2016; Wang et al. 2020a). When there is a lack of electron donor, electroactive SRB sessile cells can utilize elemental iron as an alternate electron donor, causing corrosion (Jia et al. 2019a). Corrosive APB biofilms are also a contributing factor in an acidic environment, such as bioleaching bioreactors (Ahmadi et al. 2010; Yang et al. 2020), because they release H^+ which is an oxidant (Kip and van Veen 2015).

Biofilm formation is a complex and continuous biological process (Donlan 2002; Tribedi et al. 2015; Tang et al. 2021). The formation and structure of a biofilm can be influenced by fluid flow velocity (Liu et al. 2017). The

hydrodynamics and the mechanical properties of the biofilm can impact the interactions between the bulk fluid and the attached biofilm in a flow system (Stoodley et al. 2002). The type of fluid flow is also a factor that can impact MIC. For example, compared with laminar flow, turbulent flow can lead to more severe MIC in welded joint in a pipeline (Liduino et al. 2019). Below the threshold of 3 m/s, biofilms are believed to form on pipe walls (Song et al. 2016). *Pseudoalteromonas piscicida* biofilm can induce wider and deeper cracks in horizontal flow condition than in orbital shaking condition (Moradi et al. 2019). Thus, it is necessary to test biocide mitigation of MIC in a flow system before a field trial on an actual operational process after initial biocide testing in anaerobic vials in static incubation condition.

Biocides are often used to mitigate biofilms, but large biocide dosages used in biofilm treatment suffer from high operational costs and adverse environmental impacts (Rasheed et al. 2019; Li et al. 2022). Glutaraldehyde together with tetrakis hydroxymethyl phosphonium sulfate (THPS) are the two most popular green biocides in the oil and gas industry owing to their broad-spectrum efficacy and readily biodegradable advantage (Sharma et al. 2018; Wang et al. 2020b, 2022a; Unsal et al. 2021; Kijkla et al. 2021). THPS is a broad-spectrum biocide that works by damaging cell membrane, cleaving S–S bonds in the disulfide amino acids of the cell wall (Kahrilas et al. 2015; Sharma et al. 2018). However, prolonged uses induce microbial resistance as more resistant microbes in the environment move in to fill the void left behind by vulnerable microbes.

Sessile cells embedded in a biofilm are harder to eradicate than their planktonic counterparts because biofilms can deploy several defense strategies that include

diffusional limitation, and lowered metabolic rate to minimize intakes (Li et al. 2016). Some eco-friendly chemicals such as catechin hydrate and zinc pyrithione have effective inhibition on MIC, but the industrial applications of these anti-bacterial inhibitors are still not in existence (Wang et al. 2019; Lekbach et al. 2019). Thus, a more effective and economic biocide treatment strategy is desired to treat MIC.

Using a biocide enhancer is a potential strategy in the mitigation of biofilms, because they can work synergistically with existing biocides (Kahrilas et al. 2015; Li et al. 2016). Kolodkin-Gal et al. (2010) demonstrated that several D-amino acids dispersed *Bacillus subtilis* biofilm by impacting the function of amyloid fibers. In various lab experiments, D-amino acids were shown to enhance THPS and a few other non-oxidizing biocides against mixed-culture biofilms from oilfield and cooling water towers (Li et al. 2016). Biocide enhancers can be used to improve the efficacy of a biocide in various ways and they do not have to be biocidal themselves (Wang et al. 2020b; Unsal et al. 2021). Using antimicrobial agents including antimicrobial peptides and silver nanoparticles has potential drawbacks such as a biofilm becoming possibly resistant to the agents later on because their antimicrobial actions kill the weak microbes and allow resistant microbes from the surroundings to take their place (Barapatre et al. 2016; Kumar and Sharma 2017; Di Somma et al. 2020; Hussein et al. 2020; Tan et al. 2019). Non-biocidal Peptide A is an attractive biocide enhancer candidate. It has a cyclic 14-mer sequence (cys-ser-val-pro-tyr-asp-tyr-asn-trp-tyr-ser-asn-trp-cys) with its core 12-mer sequence derived from Equinatoxin II protein, which is secreted by a sea anemone to maintain a biofilm-free exterior (Zlotkin 2016). Peptide A at a very low dosage can not only inhibit the settlement of biofilms, but also disperse pre-established biofilms in the presence of a biocidal stress as demonstrated in static vial tests (Jia et al. 2019b). The addition of 180 ppb Peptide A to 2,2-dibromo-3-nitrilpropioamide (DBNPA) showed enhanced sessile reduction and MIC abatement in 60-day incubation compared to DBNPA alone treatment in static vial tests (Wang et al. 2020b).

This work tested Peptide A at a very small dosage (100 nM, 180 ppb by mass) to enhance THPS in the mitigation of a corrosive oilfield biofilm consortium in a closed flow loop bioreactor. This work aimed to show the efficacy of THPS and the enhancement by Peptide A on THPS in the prevention of biofilm establishment as well as abatement of C1018 carbon steel MIC by an oilfield biofilm consortium in flow condition. C1018 carbon steel coupons were immersed in enriched artificial seawater (EASW) inoculated with

the biofilm consortium at 25 °C for 7 days under anaerobic condition.

Materials and methods

A recalcitrant and corrosive oilfield biofilm labelled as Consortium IIe was used in this work. Its metagenomic data are listed in Table 1. An EASW culture medium was used to culture Consortium IIe biofilm. The EASW composition was (g/L): NaCl 23.476, Na₂SO₄ 3.917, NaHCO₃ 0.192, KBr 0.096, KCl 0.664, H₃BO₃ 0.026, SrCl₂·6H₂O 0.040, CaCl₂·2H₂O 1.469, MgCl₂·6H₂O 10.610, yeast extract 1, tri-sodium citrate 0.5, sodium lactate 3.5, CaSO₄·0.5H₂O 0.1, NH₄Cl 0.1, MgSO₄·7H₂O 0.71, K₂HPO₄ 0.05, Fe(NH₄)₂(SO₄)₂·6H₂O 1.38. The purity was ≥ 99% for all the chemicals used in EASW. The initial pH of EASW was neutralized to 7.0 by adding a 5% (w/w) NaOH. EASW and lab tools such as tweezers were autoclave sterilized. EASW was then sparged with filtered N₂ to deoxygenate with the help of a gas distributor and magnetic stirring bar for at least 1 h. One hundred ppm (w/w) L-cysteine was added as an oxygen scavenger to reduce dissolved O₂ further. Peptide A (100% purity), chemically synthesized by Bachem AG (Bubendorf, Switzerland) and 75% (v/v) THPS (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in deionized water to make a stock solution that was filtered-sterilized.

C1018 carbon steel coupons with a 1.2 cm² exposed top face were tested. The metal's mass composition was (%): C 0.20, P 0.04, Mn 1.40, Cu 0.55, S 0.04, Ni 0.012, and Fe balance. The unexposed coupon faces were painted with inert Teflon. The coupons were abraded to a 600 grit finish, and then degreased with pure isopropanol and subsequently dried under UV light. The test matrix is shown in Table 2.

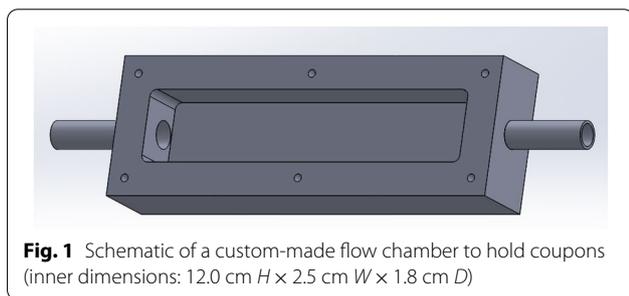
A self-fabricated acrylic flow chamber (Fig. 1) was used in this work to simulate flow condition in a re-circulating bioreactor. The carved-out inner rectangular chamber had dimensions of 12.0 cm H × 2.5 cm W × 1.8 cm D. Ten coupons were placed in the flow chamber and the

Table 1 Metagenomics of dominant microbial species in Consortium IIe

Microbe	%
<i>Garcicella</i> spp.	92.1
<i>Desulfovibrio vulgaris</i>	3.1
<i>Bacillales</i> spp.	3.0
<i>Tissierella</i> spp.	0.88
<i>Thermoanaerobacteriales</i> spp.	0.13
<i>Porphyromonadaceae</i> spp.	0.11
<i>Sphingomonas</i> spp.	0.07
Unknown	0.63

Table 2 Test matrix of biofilm prevention test in a flow loop bioreactor

Microorganism	Consortium IIe
Cultural medium	EASW
Metal	C1018 carbon steel
Treatment methods	No treatment control 50 ppm THPS 50 ppm THPS + 100 nM Peptide A 100 ppm THPS
Temperature	25 °C
Flow rate	1.2 L/h
Incubation duration	7 day
Analysis	Sessile cell count, weight loss, CLSM biofilm image, SEM corrosion pit image, pit depth



chamber was sealed with a lid with a rubber gasket fastened by six screws. Silicone glue covered the seams and tubing joints to prevent any leaks. A 10 mL Consortium IIe seed culture and biocide treatment chemicals were added to 1 L EASW medium in the 1 L anaerobic bottle that was used as the holding tank in each flow loop. Then, flow was started using a peristaltic pump (Model 7520–60, Barnant Co., Barrington, IL, USA). The re-circulation flow rate was 1.2 L/h. The entire bioreactor was placed in an anaerobic chamber (Model 818-GB, PLAS Labs, Lansing, MI, USA). This chamber was sparged with sterile N₂ for 0.75 h to achieve anaerobic condition before use. Figure 2 shows the setup for 4 independent flow loop bioreactors with a multi-channel peristaltic pump and four 1-L bottles as holding tanks.

Most probable number (MPN) method was used for cell enumerations using the modified Postgate's B (MPB) medium, standard bacterial nutrient broth, and phenol red dextrose medium for SRB, general heterotrophic bacteria (GHB) and APB, respectively. The MPN procedure can be found elsewhere (Wang et al. 2020b). Biotechnology Solutions (Houston, TX, USA) was the vendor of all these MPN liquid culture media.



After incubation, a confocal laser scanning microscope (CLSM) machine (Model LSM 510, Carl Zeiss, Jena, Germany) was employed to detect live and dead sessile cells on the coupons (Wang et al. 2022b). According to the ASTM G1–03 standard (ASTM, 2003), a fresh Clarke's solution was used then to clean the coupon surfaces completely before weighing. The coupons were then scanned under an InfiniteFocus microscope (IFM) machine (Model ALC13, Alicona Imaging GmbH, Graz, Austria) to locate MIC pits. The images of surface morphologies before and after coupon surface cleaning were examined under a scanning electron microscope (SEM) machine (Model S-2460 N, Hitachi, Tokyo, Japan).

Results and discussion

Figures 3 and 4 show the culture broth colors and coupon surfaces after the 7-day incubation with and without biocide treatment. Judging from the iron sulfide black color, with the treatment of 50 ppm or 100 ppm THPS, or the combination treatment of 50 ppm THPS + 100 nM Peptide A, the broth colors and coupon surfaces appeared less dark than without treatment (Fig. 3), indicating growth inhibition due to biocide treatment.

Figure 5 shows sessile cell counts. The no treatment coupon (control) surface had the same sessile cell count of 10⁷ cells/cm² for SRB, APB and GHB. With 50 ppm THPS in the EASW, 1-log reduction in SRB and 1-log reduction in GHB sessile cell counts were achieved, but there was no reduction in APB. With 50 ppm THPS + 100 nM Peptide A combination in EASW, an additional 1-log reduction in SRB and additional 1-log reduction in APB sessile cell counts were obtained, compared to 50 ppm THPS alone treatment.

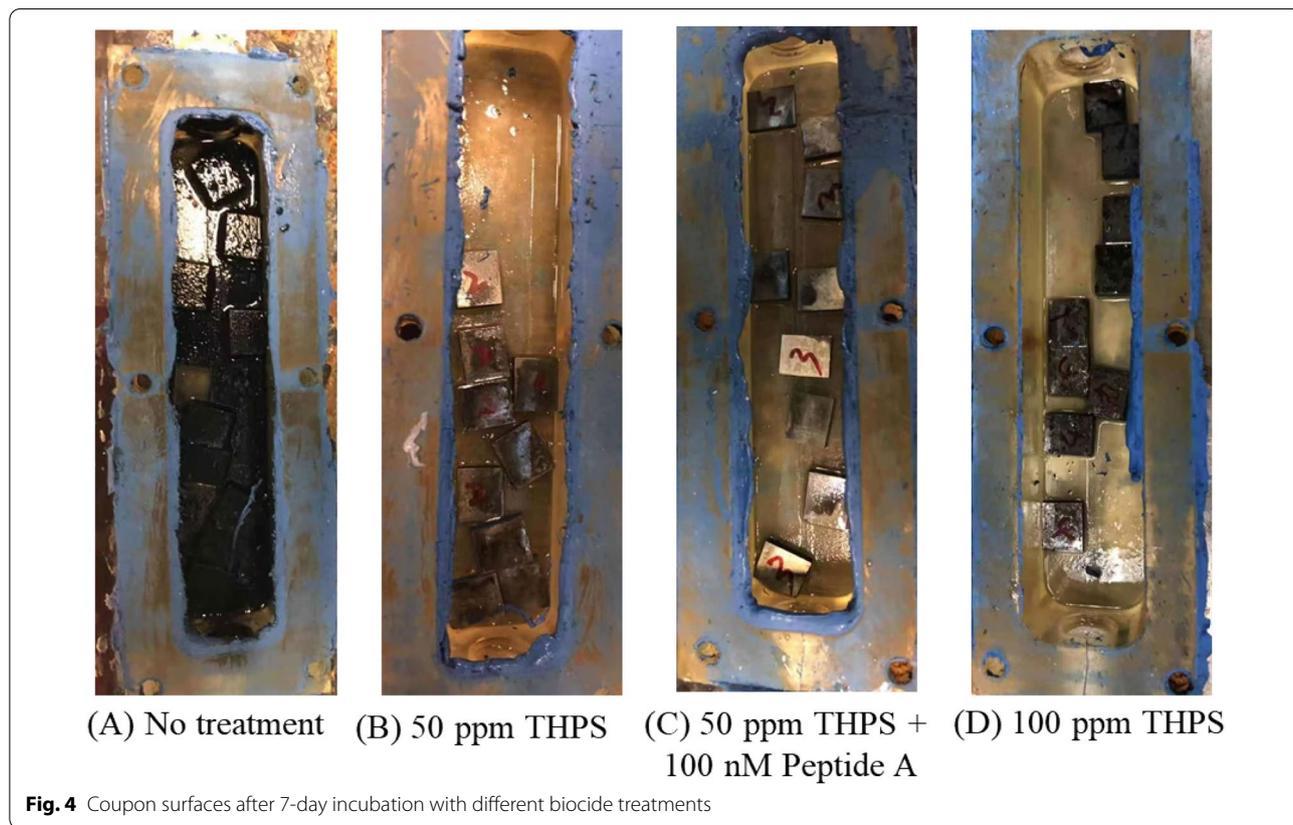
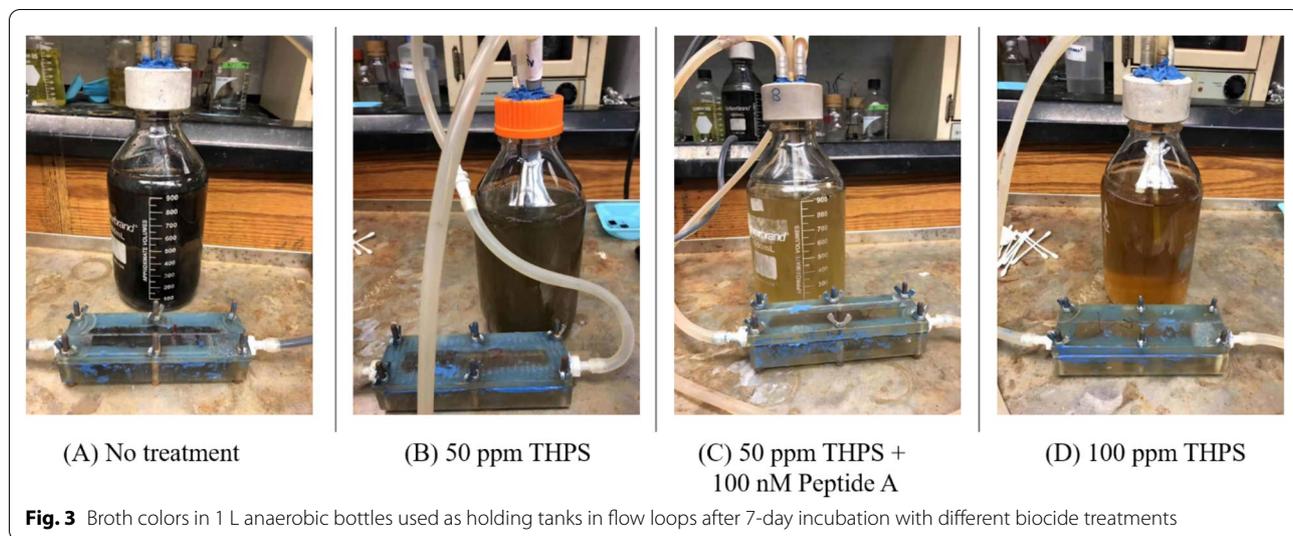
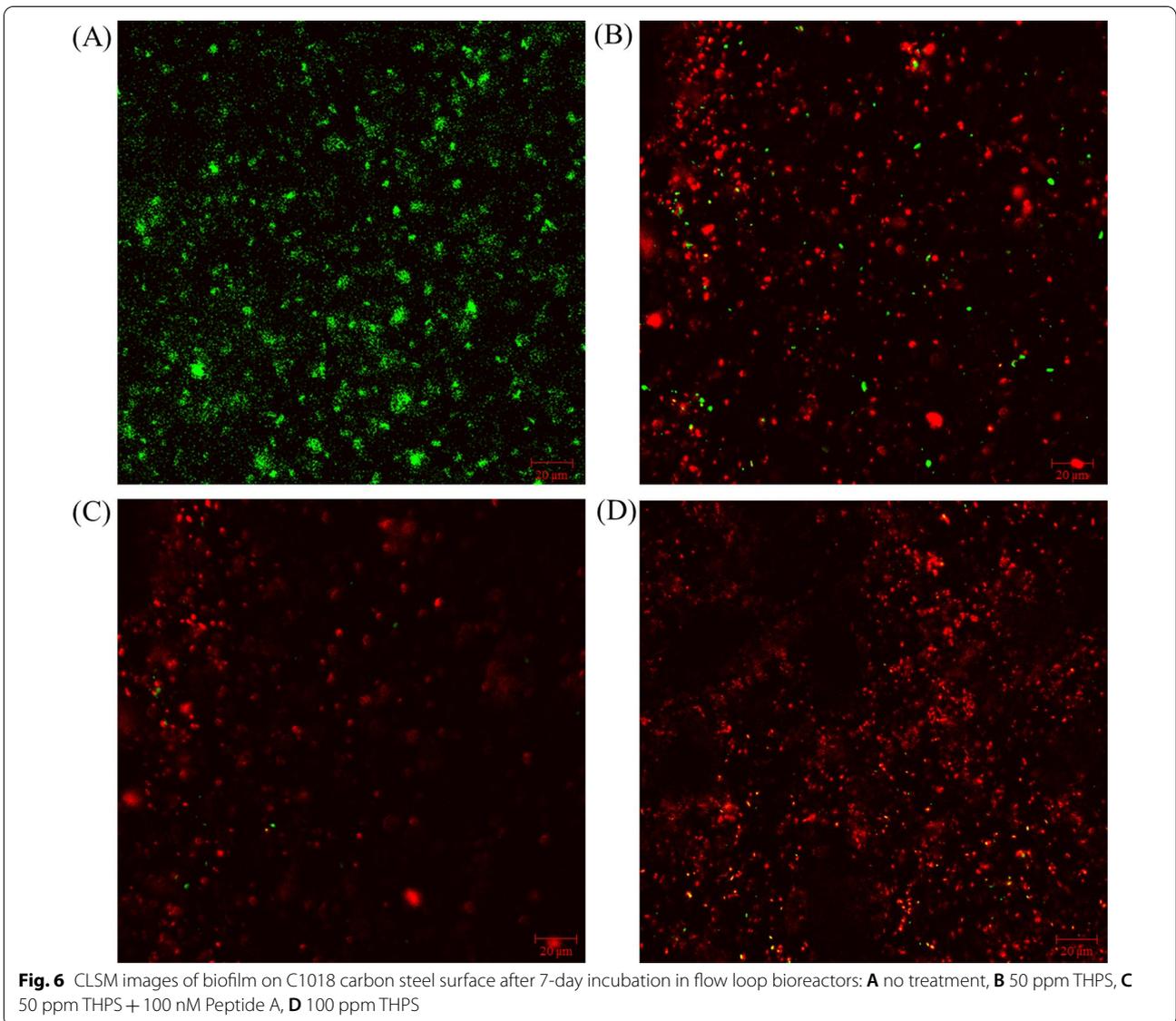
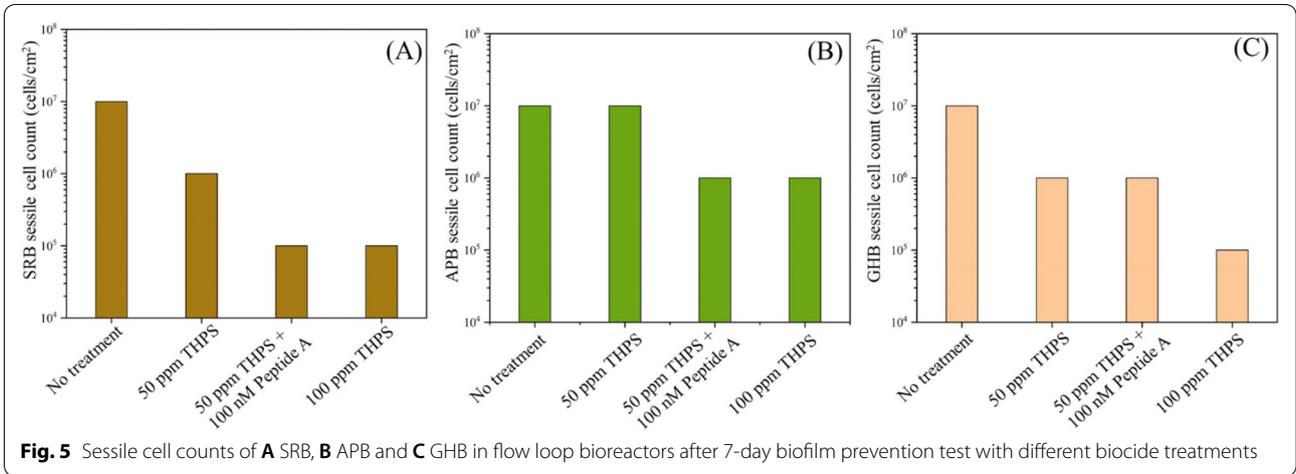


Figure 6 shows the CLSM images of the Consortium IIe biofilms on the C1018 coupons after the 7-day biofilm prevention test. Figure 6A shows that live cells (green) were numerous without biocide treatment (control). When the 50 ppm THPS was used, plenty of dead sessile cells (red) are seen, but some live cells still can be seen

in Fig. 6B. With 50 ppm THPS+100 nM Peptide A in EASW, there was less biomass on the coupon and fewer live cells, exhibiting better efficacy than 50 ppm THPS alone treatment. This outcome was similar to that from 100 ppm THPS alone treatment, but there was a noticeable difference: there were fewer attached dead cells when



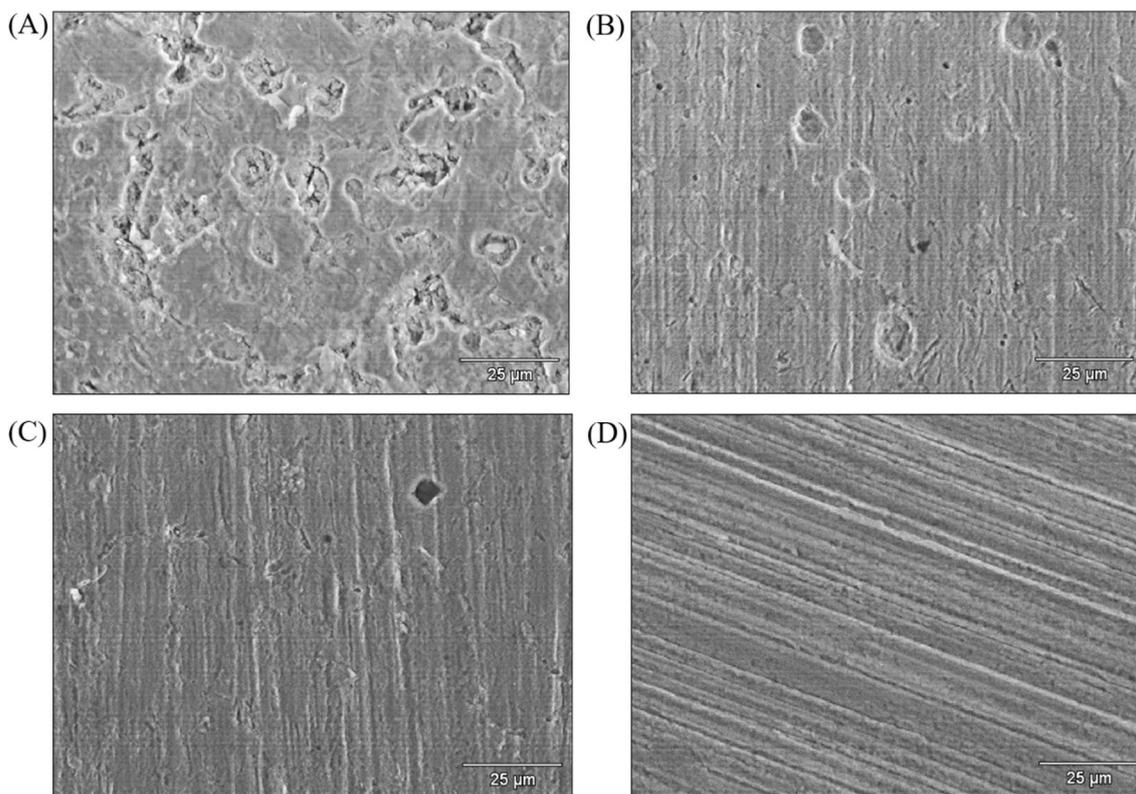


Fig. 7 SEM images of corrosion pits on C1018 carbon steel coupons after 7-day incubation in flow loop bioreactors: **A** no treatment, **B** 50 ppm THPS, **C** 50 ppm THPS + 100 nM Peptide A, **D** 100 ppm THPS

100 nM Peptide A was used as a biocide enhancer. This suggests that non-biocidal Peptide A had a biofilm dispersal effect (Jia et al. 2019b).

Corrosion pit images are shown in Fig. 7 for different biocide treatments. Figure 7A indicates that MIC pits are visible on the no treatment coupon (control), which lost parallel polishing lines due to corrosion damage. With 50 ppm THPS alone in EASW (Fig. 7B), fewer corrosion pits can be found on the coupon surfaces and polishing lines are clearly visible. The 50 ppm THPS + 100 nM Peptide A combination treatment (Fig. 7C) led to fewer and smaller corrosion pits on coupon surfaces. The pristine polishing lines are seen for the coupon with 100 ppm THPS treatment in Fig. 7D. The results of SEM pit images were consistent with the CLSM images and sessile cell count results, suggesting fewer sessile cells leading to less MIC pitting.

Figure 8 summarizes MIC weight loss data. Each weight loss data point represents the average from six replicate coupons from two replicate experimental runs. The weight loss was 1.7 mg/cm² (equivalent 0.11 mm/y uniform corrosion rate) without treatment. With 50 ppm THPS alone treatment, it reduced to

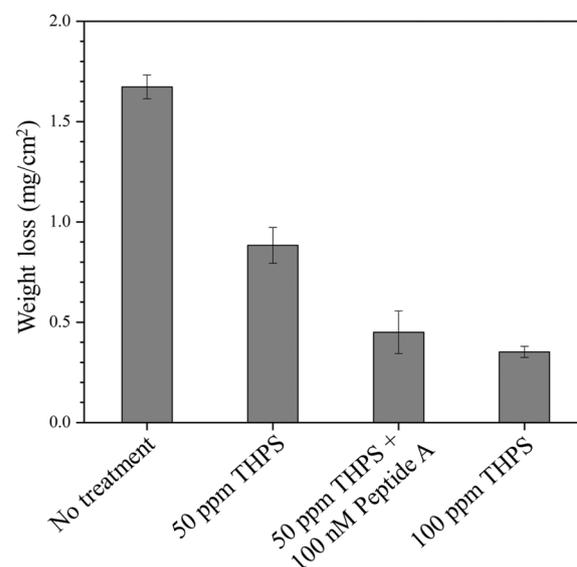
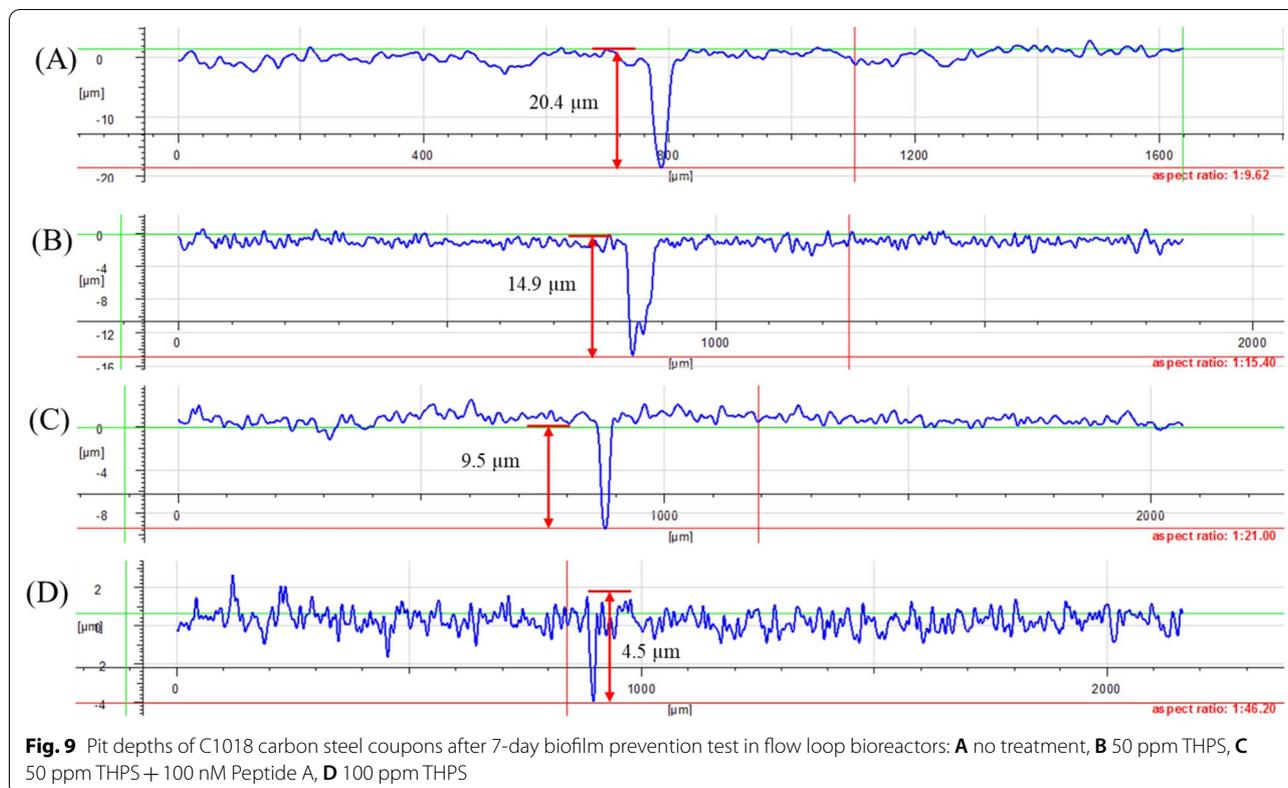


Fig. 8 Weight losses of C1018 carbon steel coupons with different biocide treatments after 7-day incubation in flow loop bioreactors (error bars represent standard deviation from three independent samples)



0.9 mg/cm² (0.060 mm/y). The 50 ppm THPS + 100 nM Peptide A combination treatment reduced the weight loss further to 0.5 mg/cm² (0.033 mm/y), which was only slightly larger than the 0.4 mg/cm² (0.027 mm/y) weight loss for 100 ppm THPS alone treatment. This means 100 nM Peptide A effectively reduced the THPS dosage from 100 to 50 ppm in terms of weight loss reduction. The 100 nM Peptide A enhancement decreased weight loss by 71% and 44% compared to no treatment (control) and 50 ppm THPS alone treatment outcomes, respectively.

Figure 9 shows the pit depths with different biocide treatments after the 7-day incubation in the flow loop bioreactors. The (maximum) pit depth for the no treatment control coupon was 20.4 µm. With 50 ppm THPS, it was lowered to 14.9 µm. After 100 nM Peptide A enhancement, the 50 ppm THPS + 100 nM Peptide A combination achieved additional 36% reduction, which lowered the pit depth further to 9.5 µm. The pit depth decreased to 4.5 µm when 100 ppm THPS was in EASW, better than the outcome with 50 ppm THPS + 100 nM Peptide A combination treatment. The mechanism of how Peptide A works as biofilm dispersal agent is still unknown, it very likely works as a signaling agent due to its ultra-low dosage (Jia et al. 2019b). Like D-amino acids, for a recalcitrant biofilm on carbon steel like the

one in this work, Peptide A requires a biocidal stress to exhibit a biofilm dispersal effect (Li et al. 2016; Jia et al. 2019b).

Conclusions

In this work, 50 ppm THPS enhanced by 100 nM Peptide A had considerably better efficacy than 50 ppm THPS alone treatment in the mixed-culture biofilm prevention in the flow system. Additional 44% reduction in weight loss and 36% abatement in pit depth were achieved with 50 ppm THPS treatment enhanced by 100 nM Peptide A. The SEM and CLSM images and sessile cell count were consistent with pit depth and weight loss data trends. The non-biocidal biocide enhancer Peptide A in the presence of a biocidal stress exhibited biofilm dispersal effect. Peptide A at a sub-ppm concentration is a promising biocide enhancer in both flow and static MIC systems.

Acknowledgements

We acknowledge the financial support from PTT Exploration and Production, Thailand and Saudi Aramco, Saudi Arabia.

Author contributions

DW: investigation, writing—original draft, methodology. TU: investigation, writing—review and editing. SK, SP, MS, and MA: project administration, funding acquisition, review and editing. DX: review and editing. TG: conceptualization, methodology, writing—review and editing, supervision, funding acquisition. All authors read and approved the final manuscript.

Funding

This work was financially supported by PTT Exploration and Production, Thailand and Saudi Aramco, Saudi Arabia.

Availability of data and materials

Not applicable.

Declarations**Ethics approval and consent to participate**

Not applicable.

Consent for publication

All the authors have read and approved the manuscript before the submission to *Bioresources and Bioprocessing*.

Competing interests

The authors declare that they have no competing interests.

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Received: 12 April 2022 Accepted: 20 May 2022

Published online: 13 June 2022

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