

**Mitigation of carbon steel biocorrosion using a green biocide enhanced by a biofilm dispersing Peptide in a flow loop with an oilfield biofilm consortium**

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**ABSTRACT**

Biocorrosion, also called microbiologically influenced corrosion (MIC), is a major threat to oil and gas pipelines. The formation and morphology of biofilms can be impacted by fluid flow. Fluid velocity affects biocide distribution and MIC. Thus, testing the efficacy of a biocide for the mitigation of MIC under flow condition is desired before a field trial after a static test in vials has confirmed its efficacy. In this work, a bench-top closed flow loop system was set up 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 in the flow path to hold coupons. Peptide A was a chemically synthesized cyclic 14-mer with its main 12-mer sequence inspired by a biofilm dispersing protein secreted by a sea anemone that has biofilm-free exteriors. The combination of 50 ppm (w/w) THPS (tetrakis hydroxymethyl phosphonium sulfate) biocide + 100 nM (180 ppb) Peptide A (biocide enhancer) resulted in extra 1-log reduction in sulfate reducing bacteria (SRB) sessile cell count and also in 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 pit depth were achieved compared to 50 ppm THPS alone treatment.

**Keywords:** Microbiologically influenced corrosion (MIC); biofilm; flow loop; biocide; biocide enhancer; Peptide

## INTRODUCTION

MIC is a major threat to oil pipelines because it reduces the service life of pipelines and can potentially leads to catatrophes.<sup>1,2</sup> Microbial communities commonly associated with pipeline corrosion include sulfate reducing bacteria (SRB), acid producing bacteria (APB), acetogenic bacteria and methanogens.<sup>3,4</sup> In a field environment, SRB, APB and other microbes often live in a synergistic biofilm consortium.<sup>5</sup> Sessile SRB are often the main culprit of MIC.<sup>6</sup> They can utilize sulfate as the terminal electron acceptor and various carbon sources and elemental iron as electron donors.<sup>7-10</sup> Corrosive APB biofilms are also a contributing factor in an acidic environment because they release  $H^+$  which is an oxidant.<sup>11</sup>

Biofilm formation is a complex and continuous process.<sup>12</sup> The formation and structure of a biofilm can be influenced by the fluid flow velocity.<sup>13</sup> 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.<sup>14</sup> The type of fluid flow is also a factor that can impact MIC. For example, compared with laminar flow, turbulent flow can lead to a higher MIC severity in welded joint in a pipeline due to increased mass transfer.<sup>15</sup> Below the threshold of 3 m/s, biofilms can form on pipe walls. The sessile cells of corrosive microbes in biofilms can cause serious corrosion.<sup>16</sup> *Pseudoalteromonas piscicida* biofilm was found to cause wider and deeper cracks in horizontal flow condition than in orbital shaking condition. The corrosion behavior of *P. piscicida* biofilm was influenced by hydrodynamic conditions.<sup>17</sup> Thus, it is necessary to test biocide mitigation of MIC in a flow system after initial biocide testing in anaerobic vials in static incubation condition has shown sufficient biocide efficacy.

Biocides are often used to mitigate biofilms, but large biocide dosages used in biofilm treatment suffer from high operational costs and increased environmental impacts.<sup>18</sup> Glutaraldehyde and tetrakis hydroxymethyl phosphonium sulfate (THPS) are the two most popular biocides in the oil and gas industry because of their broad-spectrum efficacy and readily biodegradable advantage.<sup>5,19,20</sup> Over time, an increased dosage may be needed as more resistant microbes from the surroundings fill the void left behind by vulnerable microbes.<sup>21</sup>

Sessile cells in a biofilm are far more difficult to eradicate than planktonic cells suspended in the liquid phase because biofilms can employ various defense mechanisms, including diffusional limitation, and lowered metabolic rates to reduce intakes.<sup>22</sup> Some eco-friendly chemicals like catechin hydrate and zinc pyrithione have effective inhibition behaviors on MIC, but the industrial applications of these anti-bacterials are still not in existence.<sup>23,24</sup> 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, without waiting for a new blockbuster biocide to come to the market.<sup>22,25</sup> Kolodkin-Gal et al. demonstrated that D-amino acids could disperse *Bacillus subtilis* biofilm by affecting the function of amyloid fibers.<sup>26</sup> In various lab tests, D-amino acids have been found to enhance THPS and several other non-oxidizing biocides against several biofilm consortia from oilfield and cooling water systems.<sup>22</sup> Glutaraldehyde is a potent amino acid crosslinker which makes it incompatible with amino acids and peptides.

Biocide enhancers can improve the efficacy of a biocide in different ways and they don't have to be

biocidal themselves.<sup>5,20</sup> Non-biocidal Peptide A is a promising biocide enhancer. It can not only prevent the establishment of biofilms but also disperse established biofilms in static vials.<sup>27</sup> The addition of 180 ppb (w/w) Peptide A to 100 ppm 2,2-dibromo-3-nitrilpropioamide (DBNPA) showed extra 44 % in weight loss, 43 % in maximum pit depth, and 54 % in corrosion current density, in 60-day hydrotest compared to 100 ppm DBNPA treatment alone.

In this work, Peptide A was tested at a very low concentration (100 nM or 100 ppb) to enhance THPS in the mitigation of a corrosive oilfield biofilm consortium in a closed (recirculating) flow loop. This work was designed to show the efficacy of THPS and its enhancement by Peptide A in the prevention of biofilm establishment and abatement of C1018 carbon steel MIC by an oilfield biofilm consortium in flow condition. C1018 carbon steel coupons were exposed to enriched artificial seawater (EASW) inoculated with the corrosive oilfield biofilm consortium under anaerobic condition at 25°C for 7 days. The biofilms and coupon surface morphologies were examined under scanning electron microscope (SEM), confocal laser scanning microscope (CLSM) and infinite focus microscope (IFM), respectively.

## EXPERIMENTAL PROCEDURE

A tough and corrosive oilfield biofilm consortium labelled as Consortium Ile was used in this work. Its metagenomic data are listed in Table 1. The microbes were identified using the 16S-rDNA sequencing method with QIIME 2.0.<sup>28</sup> *Garciella* spp. are nitrate- and thiosulfate-reducing bacteria which were isolated from the oilfield separator.<sup>29</sup> *Desulfovibrio vulgaris* is a well-known corrosive SRB strain.<sup>30</sup> Bacillales were investigated in MIC field.<sup>31</sup> EASW medium was used to culture Consortium Ile. The medium composition was (g/L): Na<sub>2</sub>SO<sub>4</sub> 3.917, NaCl 23.476, NaHCO<sub>3</sub> 0.192, KBr 0.096, KCl 0.664, H<sub>3</sub>BO<sub>3</sub> 0.026, SrCl<sub>2</sub>·6H<sub>2</sub>O 0.040, MgCl<sub>2</sub>·6H<sub>2</sub>O 10.610, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.469, yeast extract 1, tri-sodium citrate 0.5, sodium lactate 3.5, CaSO<sub>4</sub>·0.5H<sub>2</sub>O 0.1, NH<sub>4</sub>Cl 0.1, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.71, Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O 1.38, K<sub>2</sub>HPO<sub>4</sub> 0.05. The initial pH of the culture medium was neutralized to 7.0 by adding a 5% (w/w) NaOH solution. The culture medium and lab tools were sterilized in an autoclave for 20 min at 121°C. After autoclaving, the medium was sparged with filter-sterilized N<sub>2</sub> to deoxygenate for at least 1 h. One hundred ppm (w/w) L-cysteine was added to the culture medium as an oxygen scavenger to reduce dissolved oxygen further. Peptide A, synthesized by Bachem AG (Bubendorf, Switzerland) and THPS were dissolved in deionized water to make stock solutions which were filtered-sterilized through 0.22 µm Stericup<sup>†</sup> filters (Millipore, Bedford, MA, USA). All anaerobic manipulations were carried out in an N<sub>2</sub>-filled anaerobic chamber (Model HE-493, Vacuum Atmosphere Company, Hawthorne, CA, USA). Sigma-Aldrich (St. Louis, MO, USA) and Fisher Scientific (Pittsburgh, PA, USA) were the suppliers of chemicals used in this work.

Table 1. Metagenomics of dominant microbes in Consortium Ile

| Species                       | %    |
|-------------------------------|------|
| <i>Garciella</i> spp.         | 92.1 |
| <i>Desulfovibrio vulgaris</i> | 3.1  |
| Bacillales                    | 3.0  |
| <i>Tissierella</i> spp.       | 0.88 |
| Thermoanaerobacterales        | 0.13 |
| Porphyromonadaceae            | 0.11 |
| <i>Sphingomonas</i> spp.      | 0.07 |
| Unknown                       | 0.63 |

C1018 carbon steel coupons with a 1 cm × 1.2 cm exposed top area were used for corrosion testing. The elemental composition of C1018 carbon steel was (wt%): C 0.20, Mn 1.40, P 0.04, Cu 0.55, S 0.04, Ni 0.012, Fe balance. All other surfaces were coated with inert Teflon. Before each test, coupons were abraded with 180 and 600 grit abrasive papers progressively and then cleaned with 100% isopropanol and dried under UV light for at least 20 min. The test matrix is shown in Table 2.

Table 2. Test matrix of biofilm prevention in a flow loop

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

A self-fabricated acrylic chamber for holding coupons (Figure 1) was used in this work to simulate flow condition in a re-circulating flow loop. The carved out inner rectangular chamber had inner dimensions of 12.0 cm H × 2.5 cm W × 1.8 cm D. Ten coupons were added to the coupon chamber and the chamber was sealed using a lid with a rubber gasket fastened with six screws. Silicone glue covered the seams and tubing joints to prevent the contact between the inside fluid and the outside atmosphere. A 10 mL Consortium IIe seed culture and treatment chemicals were added to 1 L EASW medium in the 1 L (nominal) anaerobic bottle used as the holding tank in the flow loop system. Then, flow was started using a silicone tubing driven by a peristaltic pump (Model 7520-60, Barnant Co., Barrington, IL, USA). The flow rate was 1.2 L/h. The system was placed in an anaerobic chamber (Model 818-GB, PLAS Labs, Lansing, MI, USA). This anaerobic chamber was sparged with filter-sterilized N<sub>2</sub> before use to achieve anaerobic condition before use. Figure 2 shows the setup (4 independent flow systems) with a multi-channel peristaltic pump and 4 1-L bottles.

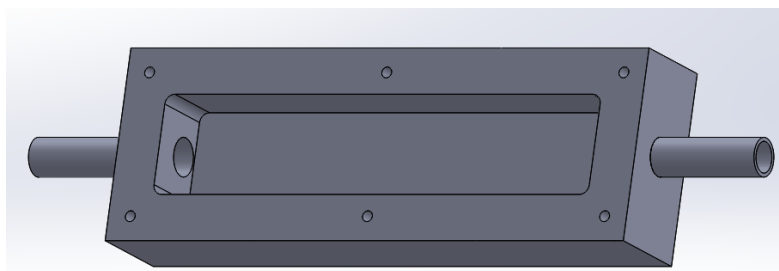


Figure 1. Schematic of a chamber for holding coupons in a flow loop.