Oil field microorganisms cause highly localized corrosion on chemically inhibited carbon steel

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Summary
Carbon steel pipelines, a means for crude oil transportation, occasionally experience highly localized perforation caused by microorganisms. While microorganisms grown in laboratory culture tend to corrode steel specimens unevenly, they rarely inflict a corrosion morphology consistent with that of pipelines, where centimetre-sized corrosion features are randomly distributed within vast stretches of otherwise pristine metal surface. In this study, we observed that corrosion inhibitors (CIs), widely used for the control of acid gas (H$_2$S, CO$_2$) corrosion in oil fields, also affect microbial growth and activity. Inhibited carbon steel resisted biofilm formation and underwent negligible corrosion (< 0.002 mm Fe$^0$ year$^{-1}$), despite 15 months of exposure to oil field waters harbouring a diverse microbiome. In contrast, physical scavenging of CI in these waters led to severe and highly localized corrosion (up to 0.93 mm Fe$^0$ year$^{-1}$) underneath biofilms dominated by methanogenic archaea and sulfate-reducing bacteria. A sharp decline in CI concentration, as well as its active components, quaternary ammonium compounds (QACs), correlated with microbial sulfidogenesis. CIs are ubiquitously present in oil field waters and play an underappreciated role in microbial corrosion mitigation. Physical and biological scavenging of CIs may create local differences in steel inhibition effectiveness and thus result in highly localized corrosion.

Introduction
In 2018, global crude oil production exceeded 100 million barrels per day for the first time in history (U.S. Energy Information Administration, 2018). The transportation of these hydrocarbons to industrial end users (e.g. refineries) requires vast infrastructure, such as buried and above-ground pipelines. Carbon steel is the preferred manufacturing material for such pipelines, due to its excellent mechanical properties and low cost (Ahmad, 2006). However, carbon steel is also inherently prone to corrosion and the integrity of ferrous infrastructure requires careful management in order to guarantee long-term operability and to prevent the release of hazardous liquids to the environment (Ahmad, 2006).

At oil production sites, a primary means of corrosion mitigation is the use of corrosion inhibitors (CIs; Sanyal, 1981; Kermani and Morshed, 2003; Achour and Kolts, 2015). These oil field chemicals ward against the corrosive effects of the acid gases CO$_2$ and H$_2$S, which are soluble in water and can be abundant in petroleum reservoirs (Nešić, 2007). Most CIs are surfactant-like organic molecules that chemisorb to steel and form a physical barrier between the metal and its corrosive environment (Migahed and Al-Sabagh, 2009). They owe this functional property to their molecular structure; a surface-active head group and hydrophobic aliphatic tail structure allows the CI to bind to metallic surfaces and form a persistent film that repels hydrophilic molecules (Sanyal, 1981; Migahed and Al-Sabagh, 2009). Commercially available products for inhibition of acid gas corrosion are often chemical mixtures that contain the surfactant-like active ingredient(s) blended into solvents and synergistic chemistries. Commonly used active ingredients include quaternary ammonium compounds (QACs), imidazoles, imidazolines, amines and phosphate esters (Dariva and Galio, 2014). The inhibition of CO$_2$ (carbonic acid) corrosion with CI can be depicted as follows:

$$\text{Fe}^0 + 2\text{H}_2\text{CO}_3 + \text{CI} \rightarrow \text{Fe}^{2+} + 2\text{HCO}_3^− + \text{H}_2$$  \hspace{1cm} (1)

While carbonic acid tends to be less problematic in waters at pH > 7 where it gets rapidly deprotonated and protective iron carbonate (FeCO$_3$) films are more likely to develop on carbon steel surfaces, carbonic acid corrosion needs to be adequately inhibited under the (slightly)
acidic conditions that prevail in most production systems. Typical injection rates in oil fields are 10–300 mg Cl l$^{-1}$ of total fluid (crude oil + water; Palmer et al., 2004).

In addition to dissolved acid gases, the oil field microbiome poses a threat to materials integrity (Duncan et al., 2009; Vigneron et al., 2016; Vigneron et al., 2017; Fig. 1). The infrastructure used for the production, processing and transportation of crude oil teems with microbial life, and many different microorganisms have been demonstrated to affect the corrosion of steel, a phenomenon termed microbially influenced corrosion (MIC). Two physiological groups that have received particular attention in the context of MIC are the sulfate-reducing bacteria (SRB) and methanogenic archaea, owing to their prevalence in corroding infrastructure and the ability of some isolates to inflict severe metal damage in laboratory experiments (Mori et al., 2010; Uchiyama et al., 2010; Enning et al., 2012; Enning and Garrels, 2014). Most crude oil-associated anoxic waters are replete with readily available organic and inorganic electron donors for anaerobic microbial metabolism (Magot et al., 2000). This can be problematic if the microbial degradation of these compounds is coupled to the production of corrosive metabolites such as reduced sulfur species (Lee et al., 1995; Enning and Garrels, 2014; Lahme et al., 2019) or nitrite (Drenen et al., 2014; Lahme et al., 2019). Corrosion that is caused by microbial metabolites is sometimes referred to as chemical microbially influenced corrosion (CMIC; Enning et al., 2012). CMIC may, for instance, result from the incomplete oxidation of propionate by SRB and the concomitant generation of sulfide (Eq. 2), which is corrosive to steel (Eq. 3).

\[
4\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{SO}_4^{2-} \rightarrow 4\text{CH}_3\text{COO}^- + 4\text{HCO}_3^- + 3\text{HS}^- + \text{H}^+ \tag{2}
\]

\[
\text{Fe}^0 + \text{HS}^- + \text{H}^+ \rightarrow \text{FeS} + \text{H}_2 \tag{3}
\]

Another corrosion mechanism that has come to prominence in recent years is the so-called electrical microbially influenced corrosion (EMIC; Dinh et al., 2004; Enning et al., 2012). Some microorganisms can grow lithotrophically with metallic iron (Fe$^0$) as their sole electron donor in laboratory experiments. These peculiar isolates of SRB and methanogenic archaea derive energy from the coupling of Fe$^0$ oxidation to the reduction of sulfate (Eq. 4) and CO$_2$ (Eq. 5), respectively.

\[
\text{Fe}^0 + \text{SO}_4^{2-} + \text{H}_2 \rightarrow \text{FeSO}_4 + \text{H}_2 \tag{4}
\]

\[
\text{CH}_4 + 2\text{H}_2 \rightarrow \text{CH}_4 \tag{5}
\]

**Fig. 1.** Examples of highly localized corrosion in crude oil transmission pipelines. All images depict internal corrosion features on the bottom of carbon steel pipelines. White arrows in B and C indicate full wall penetration. A: bar = 2 cm. B: bar = 1 cm. C: bar = 1 cm. D: bar = 1 cm. E: bar = 1 cm.
4Fe⁰ + SO₄^{2−} + 3HCO₃⁻ + H₂O → FeS + 3FeCO₃ + 5OH⁻  
(4)

4Fe⁰ + 5HCO₃⁻ + 2H₂O → 4FeCO₃ + CH₄ + 5OH⁻  
(5)

The responsible microorganisms, many of which also grow on solid-state electrodes (Beese-Vasbender et al., 2015a,b; Deutzmann and Spormann, 2017; Deng et al., 2018), supposedly utilize iron-derived electrons for their metabolism through redox-active proteins that are in electrical short-circuit with the metal. SRB are thought to achieve this through outer membrane cytochromes (Dinh et al., 2004; Beese-Vasbender et al., 2015a,b; Deng et al., 2018; Tang et al., 2019), while methanogenic archaea oxidize iron through extracellular hydrogenases to a similar effect (Deutzmann et al., 2015; Tsurumaru et al., 2018).

It is a widely held conception in the oil and gas industry that the formation of biofilms is a prerequisite for MIC (Skovhus et al., 2017); hence mitigation of MIC in pipelines focuses on the removal and inactivation of steel-attached microorganisms, which is achieved through mechanical cleaning (‘pigging’) and periodic biocide application, respectively. Intriguingly, microbial corrosion tends to be highly localized in carbon steel pipelines, particularly in those that are downstream of processing facilities which remove much of the oil-associated natural gas (including CO₂) and water. Microorganisms typically corrode steel specimens unevenly (Enning et al., 2012) and may cause micrometre-scale pitting in laboratory experiments (Starosvetsky et al., 2000; Chen et al., 2015a,b). However, such studies come short at explaining the patchy occurrence of MIC in actual pipelines, where millimetre- to centimetre-sized localized corrosion features are found within swathes of otherwise virtually pristine metal surface (see Fig. 1 for examples).

Corrosion inhibitors (CIs) have received little attention in the context of MIC, despite their ubiquity in crude oil production, transportation and storage facilities (Duncan et al., 2014). We speculated that CIs, which are surface-active and toxic, have a major yet widely overlooked impact on oil field biofilm formation and MIC. We therefore studied the effects of CI on biofilm ecology, microbial activity and corrosion in laboratory experiments with produced waters and microorganisms from West African oil field pipelines.

Results and discussion

Corrosion inhibitors influence biofilm formation and MIC in West African produced water

As produced water gravity separates from crude oil in transmission systems, MIC is usually observed at the steel–water interface along the bottom of pipelines. While the settled water can contact vast stretches of metal, the occurrence of metal damage tends to be highly localized and at seemingly random locations (Fig. 1). We suspected that CIs, which are amply used in crude oil production facilities for the inhibition of acid gas corrosion, coincidentally also influence the extent and morphology of MIC. To test this hypothesis, we collected oil and associated produced water from a production site in West Africa which uses CI and has a documented history of microbial corrosion (Keasler et al., 2010). Once received at the laboratory, oily anoxic produced water was separated and transferred into nine butyl rubber-stoppered 1 l glass bottles containing vertically mounted corrosion coupons (microcosms, Fig. S1). This water contained a residual CI concentration of 3−5 mg l⁻¹ in the bulk liquid. In order to study the effect of CI on biofilm formation and corrosion, we lowered the concentration of CI in a sub-set of the microcosms by adding sterilized laboratory-grade sand to five of the nine bottles. Sand is known to adsorb CI in oil field settings (Horsup et al., 2007) and effectively lowered CI concentrations in this study (Fig. S2). The two sub-sets of microcosms developed markedly different appearances over the course of the 15 months experiment. While there was no physical contact between the mounted steel coupons and the added sand that accumulated on the glass bottoms, metal coupons started blackening within weeks and were covered in millimetre thick, dark deposits by the end of the experiment. By contrast, steel coupons in the unaltered (no sand) microcosms remained clean and of metallic appearance during the entire 15 months. Further analysis of the steel specimens unveiled profound differences in metal integrity (Figs 2A and S3). Deep and highly localized corrosion features were observed on coupons from microcosms with sand (Fig. S3), which had a lowered residual CI concentration of 1.2 ± 0.6 mg l⁻¹ (n = 5). Interpolated pitting (localized) corrosion rates in these bottles averaged 0.33 ± 0.07 mm Fe⁰ year⁻¹ (n = 15), and rates as high as 0.93 mm Fe⁰ year⁻¹ were observed on some coupons (Fig. 2C). Microbial corrosion of this magnitude would reduce the service life of a typical carbon steel pipeline to less than 10 years. By contrast, there was no pitting corrosion on coupons in the unaltered (no sand) bottles with higher residual CI concentrations (3.1 ± 0.4 mg l⁻¹; n = 4). In addition, Fe⁰ weight loss was negligible (0.0018 ± 0.0004 mm Fe⁰ year⁻¹; n = 12) and almost 50 times lower than in bottles with sand (0.088 ± 0.021 mm Fe⁰ year⁻¹; n = 15, Fig. 2C).

The observed differences in corrosion called for the analysis of biofilms that had developed under the respective conditions with and without CI scavenging. Intriguingly, there was little biomass on steel coupons in bottles without sand (high CI); these biofilms contained...
100 times fewer bacterial and archaeal 16S rRNA gene copies cm$^{-2}$ than biofilms grown in bottles with sand (Fig. 2C). The residual concentration of surface-active CI in the West African produced water had apparently hindered biofilm formation on tested steel specimens over more than one year in laboratory studies. The observed differences in biofilm formation were particularly interesting as there was no distinct effect of CI on planktonic cell numbers in the two sets of microcosms (Fig. S4A). The numbers of planktonic microorganisms were in fact comparable in all bottles ($4.4 \times 10^6$ – $1.7 \times 10^7$ gene copies ml$^{-1}$), i.e. unaffected by CI concentration in the bulk fluid. As negatively charged steel surfaces attract the positively charged surfactant-type CIs, it is expected that CIs are more aggregated and present at higher volumetric concentrations in the direct vicinity of the metal.

Fig. 2. Highly localized corrosion of carbon steel coupons in laboratory experiments with oily produced water or synthetic produced water medium containing oil field microorganisms. A. Photographs (top row) and surface depth profile (bottom row) of cleaned corrosion coupons exposed to anoxic oily produced water (PW) for 15 months. One set of coupons ($n = 15$) was incubated in microcosms containing PW in which corrosion inhibitor (CI) concentrations had been lowered through sand addition. Another set of coupons ($n = 12$) was incubated in PW containing residual CI. One representative coupon is shown for each condition. Bar = 1 cm. B. Corrosion coupons exposed for 3 months to oil-free, synthetic produced water medium containing different concentrations of CI ($n = 3$ for each condition). Depth profiles for $1/4$ of the surface of one representative coupon per condition are shown. Bar = 1.25 cm. White arrows denote localized corrosion features. C. Interpolated annualized weight loss-based and localized profilometry-based corrosion rates in produced water cultures ($n = 15$ with sand, $n = 12$ without sand), along with corresponding biofilm cell count ($n = 5$ with sand, $n = 4$ without sand). D. Extrapolated annualized weight loss-based and localized corrosion rates in synthetic produced water medium containing different concentrations of CI, along with corresponding biofilm cell count ($n = 3$). Extrapolated annualized weight loss-based corrosion rates in sterile synthetic produced water medium are also shown ($n = 1$).

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Fig. 3. Steel-attached biofilm and planktonic microbial communities in anoxic microcosms with oily produced water and carbon steel coupons at the end of the 15 months experiment. One set of microcosms ($n = 4$) was incubated without sand, with produced water still containing $3.1 \pm 0.4$ mg CI l$^{-1}$ at test end. In the other set of microcosms ($n = 5$), residual CI concentrations had been lowered through addition of sand to $1.2 \pm 0.6$ mg l$^{-1}$.

A. Principal component analysis of microbial community composition in steel-attached biofilms (sessile) and free produced water (planktonic).
B. Sessile bacterial and archaeal microbial community composition of biofilms collected from steel coupons.
C. Planktonic bacterial and archaeal microbial community composition in microcosm produced waters. (B) and (C) depict families which account for $\geq2.5\%$ of total reads (average of biological replicates) in at least one of the four data sets (sand, no sand, sessile, planktonic).
Corrosion inhibitors impact microbial activity
compared to the bulk fluids (Zhu et al., 2017). This may explain the profound differences between biofilm and planktonic cell viability in cultures without sand.

Furthermore, 16S rDNA amplicon sequencing revealed similarities in planktonic microbial community composition between the nine microcosms at the end of the experiment regardless of CI concentration (Fig. 3A and C). Steel-attached biofilms in incubations with lowered CI concentrations, on the other hand, were dominated by markedly different microorganisms (Fig. 3A and B). Archaeal Methanobacteriaceae accounted for 30.3 ± 3.4% of the microbial population covering corroded steel surfaces (with sand, low CI), but were only minor community members (1.8 ± 1.5%) of the overall less abundant biofilm on uncorroded coupons (no sand, high CI). It is tempting to speculate that these steel-attached archaea may have caused much of the observed metal damage. Recently, the genome of one species within the Methanobacteriaceae was shown to encode a corrosive extracellular [NiFe]-hydrogenase (Tsurumaru et al., 2018), while other strains have been found to rapidly corrode Fe0 (according to Eq. 5) in laboratory experiments (Dinh et al., 2004). In this experiment, Fe0 served as a solid-state electron donor and may have enriched for sessile microorganisms that are capable of an Fe0-based metabolism. However, steel-derived electrons remained inaccessible in the set of microcosms that contained higher concentrations of CI. Only a small number of microorganisms attached to the chemically inhibited steel (4.8 × 10^5 ± 1.6 × 10^5 gene copies cm^-2), and these microorganisms resembled the microbial makeup of the produced water (Figs 2C and 3A–C). Members of the Methanosetaeaceae and Desulfobulbaceae, for instance, can degrade acetate and propionate which were present in the West African produced water, but contained only acetate (12.1 mM) and propionate (1.6 mM) as organic electron donors. TriPLICATE 2 l microcosms containing carbon steel coupons were amended with CI at 0, 3, 5 and 10 mg l^-1 and inoculated with an enrichment culture from the oil field. These experimental conditions were selected to model concentrations of CI previously observed in bottle tests with actual produced waters. The commercial CI which was used in the West African oil field and in this laboratory study, contains quaternary ammonium compounds (QACs) with hydrophobic aliphatic chain lengths between C12 and C16. Besides their use in CIs, QACs find application as organic disinfectants in other industries and are, for example widely used in consumer care products (Tezel and Pavlostathis, 2015). QACs are cationic surfactants that owe their antimicrobial properties to the disruption of a cell’s physical and ionic stability (Wessels and Ingmer, 2013). Indeed, we confirmed that as little as 3 mg l^-1 of this QAC-containing CI effectively prevented sulfidogenesis and methanogenesis in Fe0-containing planktonic cultures of the oil field enrichment (Fig. S5). The response in Fe0-containing microcosms, on the other hand, was different. Steel-attached biofilm grew despite the addition of 3 mg l^-1 or 5 mg l^-1 CI and reached comparable cell counts as the CI-free control (4.6 × 10^7 ± 4.0 × 10^7 gene copies cm^-2; Fig. 2D). Furthermore, biofilms grown in synthetic produced water offer two mechanistic explanations for our observations. First, the film-forming CIs protected steel effectively against the corrosive metabolite sulfide (e.g. from the metabolism of Desulfobulbus sp.; see Eqs. 2 and 3), and hence mitigated CMIC. Second, CIs exhibited a surface toxicity effect that prevented the growth of steel-attached corrosive microorganisms capable of EMIC, such as Methanobacterium sp. In microcosms where sand had been added to physically scavenge some of the CI, chemical inhibition was reduced in its effectiveness and highly localized corrosion on otherwise pristine steel surfaces resulted from insufficient chemical protection (Figs 2A and S3). However, while adequately simulating oil field MIC, the use of oily produced water and physical scavenging of CI may have potentially introduced experimental artefacts that complicate the interpretation of our results. We therefore investigated the activity of oil field microorganisms in the presence of CI under more defined conditions, using synthetic growth media.

**Under-dosing of CI led to microbi ally influenced, highly localized corrosion on carbon steel**

In order to further study the impact of CI on microbial activity, we incubated steel specimens in microcosms with synthetic produced water medium for three months. This medium was modelled after the West African produced water, but contained only acetate (12.1 mM) and propionate (1.6 mM) as organic electron donors. Triplicate 2 l microcosms containing carbon steel coupons were amended with CI at CI and reached comparable cell counts as the CI-free control (4.6 × 10^7 ± 4.0 × 10^7 gene copies cm^-2; Fig. 2D). Furthermore, biofilms grown in synthetic produced water
medium with 3 mg l\(^{-1}\) or 5 mg l\(^{-1}\) Cl contained similar numbers of microorganisms as biofilms grown in actual produced water with lowered CI concentration (sand addition; compare Fig. 2C and D). Addition of 10 mg CI l\(^{-1}\), however, profoundly limited formation of biofilm (3.1 \(\times\) 10\(^4\) \pm\) 1.0 \(\times\) 10\(^4\) gene copies cm\(^{-2}\)). Chemical inhibition reduced both, abiotic and microbially influenced oxidation of Fe\(^0\), with at least threefold lower coupon weight loss observed in microcosms with CI compared to the CI-free controls (Fig. 2D). Overall, the formation of biofilm on steel coupons in these tests only moderately increased coupon weight loss compared to sterile controls (an increase of 33–75%). However, this microbially influenced increase in corrosion occurred in a highly localized manner on chemically inhibited steel surfaces in cultures with a starting concentration of 3 mg l\(^{-1}\) or 5 mg l\(^{-1}\) Cl (Fig. 2B). Technically relevant localized metal damage was observed on these coupons, and corrosion rates (0.39 \(\pm\) 0.05 mm Fe\(^0\) year\(^{-1}\); Fig. 2D) were in fact comparable to those in long-term produced water incubations with lower CI concentrations (Fig. 2C). We attributed the highly localized corrosion to microbial activity, as control incubations with CI under sterile conditions were devoid of such corrosion features (Fig. S6).

Addition of CI effectively diminished corrosion and the formation of molecular hydrogen in sterile control incubations (Eq. 1, Figs 2D and S7B). Despite reaching similar biofilm cell counts by the end of the experiment, microbial activity throughout the experiment was affected by the addition of CI at 3 mg l\(^{-1}\) or 5 mg l\(^{-1}\). Addition of these chemicals delayed microbial sulfate reduction and methanogenesis, as well as the microbial oxidation of H\(_2\), propionate and acetate compared to CI-free controls (Fig. S6). The delay may be best explained by an impediment of microbial metabolism due to sublethal concentrations of the added organic disinfectants. The inhibitory effect of QACs on methanogenic archaea in wastewater treatment plants and biogas reactors has been observed elsewhere (Garcia et al., 1999; Tezel et al., 2006). At 10 mg CI l\(^{-1}\), microbial activity was reduced to a minimum and only apparent from the consumption of small quantities of cathodic hydrogen (from Eq. 1; Fig. S7A).

Biofilms were largely composed of sulfate-reducing Desulfovibrionaceae and Desulfobulbaceae, along with a number of facultatively anaerobic microorganisms within the families Pseudomonadaceae, Bacillaceae and Deferribacteraceae (Fig. S9A). The synthetic produced water further contained large proportions of Desulfobacteraceae and Methanosaetaceae in several of the incubations (Fig. S9B). We did not, however, observe in any of these incubations members of the Methanobacteriaceae, for which we had previously speculated an involvement in EMIC and which were abundant in tests with actual produced water (Fig. 3). The detection of delta-proteobacterial SRB and acetoclastic methanogens (Methanosaetaceae) was consistent with the observed consumption and production patterns of propionate, acetate, sulfide and methane (Fig. S8A–D). The role of other detected microorganisms was less apparent and could not be inferred from the monitored metabolites.

**Oil field microorganisms influence the performance and concentration of corrosion inhibitors**

Laboratory testing with synthetic produced water and the West African enrichment culture supported our hypothesis that certain CIs can prevent biofilm formation and MIC on carbon steel. Additionally, we were able to reproduce the observation that inhibited carbon steel can become subject to highly localized microbial corrosion if an insufficient quantity of CI is present. In the absence of CI, the oil field culture rapidly performed incomplete oxidation of propionate and reduced sulfate (according to Eq. 2; Fig. S8A–C). The resultant sulfide reacted with steel (Eq. 3) and/or precipitated with ferrous iron from CO\(_2\) corrosion (Eq. 1) as FeS, and dissolved sulfide concentrations declined steadily over the course of the experiment (Fig. 4A). We attributed the observed incremental metal loss in the presence of the oil field microorganisms to CMIC, as the detected quantities of sulfide (up to 0.6 mM) were sufficient to explain the surplus of iron oxidation (according to Eq. 3), compared to sulfide-free sterile controls. The onset of sulfidogenesis was delayed in incubations with 3 mg l\(^{-1}\) or 5 mg l\(^{-1}\) CI, and sulfide concentrations remained steady during the course of the experiment due to the chemical inhibition of (biogenic) H\(_2\)S corrosion (Fig. 4B and C). Intriguingly, CI concentrations declined in these microcosms, a phenomenon that was not observed in sterile controls and was, as such, attributed to microbial activity. The decline in detectable CI in triplicate microcosms coincided precisely with the onset of sulfide formation in these cultures (Fig. 4B and C, shaded areas). Our CI detection methodology is based on the formation of a chloroform-soluble, coloured complex between methyl orange and cationic surfactants (such as QACs; Wang and Langley, 1975). We excluded both, an interference of dissolved sulfide with the assay and a reaction of dissolved sulfide with CI as potential explanations for this observation. CI concentrations remained steady in sterile laboratory experiments containing sulfide (Fig. S10A), with minor decreases in CI concentrations best explained through formation of CI-binding iron sulfide particles from traces of dissolved iron (7.5 µM) in the culture medium. Negatively charged surfaces such as those generated through precipitation of FeS would be expected to bind cationic surfactants and thereby reduce detectable CI concentrations. It is conceivable that biogenic H\(_2\)S from
planktonically grown SRB caused the highly localized corrosion on insufficiently inhibited steel (i.e. at 3 mg l\(^{-1}\) or 5 mg l\(^{-1}\) CI) and that the resultant larger quantities of FeS particles (from Eq. 3) adsorbed CI in these incubations. This may have then paved the way for microbial attachment and growth of sessile microorganisms, thereby further exacerbating MIC. Alternatively, SRB might have influenced CI concentrations more directly. We observed a decline of CI concentrations also in Fe\(^0\)-free anaerobic incubations (Fig. S10A), and QACs may have been hydrolysed or sequestered by this sulfidogenic oil field enrichment culture. The anaerobic degradation of QACs is believed to be a slow process in natural and engineered environments (Zhang et al., 2015). On the other hand, it may be worth considering that this enrichment culture has been obtained from an oil field with continuous exposure to this particular chemical over years, and as such may have adapted. Under aerobic conditions, degradation of QACs can be swift and Pseudomonas spp. play a prominent role as they perform the chemical activation and \(\beta\)-oxidation of the aliphatic side chains (Tezel and Pavlostathis, 2015). When added to an oxygenated artificial seawater medium with a marine inoculum, we observed biological degradation of 20 mg l\(^{-1}\) of this CI within just 3 weeks (Fig. S11). Pseudomonads are commonly observed in oil fields (Li et al., 2014; Vigneron et al., 2016) and were also detectable in our mixed microbial communities (Fig. S9), but their potential involvement in the observed drop of CI concentration remains elusive as neither oxygen nor nitrate was present as electron acceptor for these facultatively anaerobic microorganisms in our experiments.

In order to evaluate if also individual, commonly used active ingredients of CIs are affected by microorganisms in a similar manner as the tested chemical cocktail, we conducted experiments with benzyldimethyl-n-dodecyl-ammonium chloride (a C\(_{12}\)-QAC) and benzyldimethyl-n-hexadecylammonium chloride (a C\(_{16}\)-QAC). In experiments with 2 mg l\(^{-1}\) of the C\(_{12}\)-QAC, its concentration decreased and became undetectable within three weeks in the presence of steel coupons and the oil field enrichment culture (Fig. S12A). Similarly, the concentration of C\(_{16}\)-QAC dropped below that of a sterile control, albeit not to zero, within the test duration (Fig. S12B). It is possible that the increased chain length of C\(_{16}\)-QAC made it less biodegradable (Garcia et al., 1999). Again, localized MIC was observed on inhibited steel, yet only in one of the two duplicate cultures with C\(_{12}\)-QAC (Fig. S12C).

**Towards an understanding of oil field biofilm formation and MIC on chemically inhibited steel**

The formation of biofilm on metallic surfaces can lead to corrosion (Fig. 5A). In crude oil transmission pipelines,

![Fig. 4. Dissolved sulfide (dashed lines) and corrosion inhibitor (CI) concentration (bold lines) in biological triplicate cultures of oil field microorganisms in synthetic produced water medium containing carbon steel coupons. CI concentration in sterile controls is also shown. Sulfide was not detected in any of the sterile controls (data not plotted).](image-url)

A. CI-free control cultures.
B. Cultures with addition of 3 mg CI l\(^{-1}\) at test start.
C. Cultures with addition of 5 mg CI l\(^{-1}\) at test start.
D. Cultures with addition of 10 mg CI l\(^{-1}\) at test start. Shaded areas in (B) and (C) highlight sulfidogenesis and biologically mediated decrease of CI concentration in these actively growing oil field cultures.
such MIC tends to occur in a highly localized manner (Fig. 1). The reasons for the occurrence of randomly distributed corrosion features on otherwise uncorroded steel surfaces, however, have remained enigmatic. In this study, we produced evidence that CIs can confer long-term protection of carbon steel through prevention of biofilm formation and protection against corrosive metabolites, such as H₂S (Fig. 5B IV and V). QAC-containing CIs affect microbial metabolism in a concentration-dependent manner. While at high concentrations QACs are toxic to microbial life, their mode of action at sublethal concentrations is complicated and includes multiple processes such as loss of membrane osmoregulation, dissipation of the proton-motive force, inhibition of respiratory enzymes as well as oxidative stress which can ultimately lead to mutations and gene transfers (Tezel and Pavlou, 2015). We observed retarded microbial growth at concentrations of 3 mg l⁻¹ or 5 mg l⁻¹ of this particular QAC-containing CI (Figs 4 and S8A–D). At higher concentrations, CI can form an apparently impenetrable layer on carbon steel that prohibits microbial colonization of the metallic substratum through disruption of cell membrane functionality (Fig. 5B V). The effect of CI on cellular metabolism and viability may be particularly pronounced for microorganisms at the steel–water interface, as this is where CI concentrations will be highest (Zhu et al., 2017). We used biofilm staining with the fluorescent dyes SYTO9® and propidium iodine to visualize that the membrane integrity of microorganisms attached to chemically inhibited steel was in fact compromised.
(Fig. 5C and D). CIs impact the oil field microbiome, and can in extreme cases profoundly limit the ability of microorganisms to attach to carbon steel surfaces within the vast oil production and transportation infrastructure (Fig. 5B and D).

It is conceivable that inhibited and un-inhibited steel surfaces coexist in the same pipeline, thereby explaining local differences in corrosion. Analysis of biofilm from a corroded crude oil transmission pipeline in North America supported this assumption (Fig. 1B). The corrosion feature in the 6 o’clock position of this pipeline contained mature biofilm (3.1 × 10^9 16S rRNA gene copies cm^-²), while there were three orders of magnitude fewer cells (2.4 × 10^5 16S rRNA gene copies cm^-²) on the virtually uncorroded pipeline surface in its immediate proximity. These numbers were in fact remarkably similar to those observed in our long-term laboratory tests with actual and synthetic West African produced waters (Fig. 2C and D). It is currently unknown whether the occurrence of localized MIC on chemically inhibited carbon steel is truly random. The initiation of pitting corrosion by acid gases on chemically inhibited steel has been attributed to local differences in metal microstructure (Hayden et al., 2019), and this may also apply to microbiological instances of corrosion (Walsh et al., 1993). However, highly localized MIC on chemically inhibited carbon steel in pipelines may also be explained through heterogeneous sand deposition which may locally scavenge CI (Pandarinathan et al., 2013; compare also Fig. S2) and thereby initiate MIC underneath these deposits. Corrosion under deposits is widely observed in oil fields and has in some instances been linked to MIC (Vera et al., 2012). Crude oil transmission pipelines could be particularly prone to the effects of MIC on chemically inhibited steel. First, MIC is typically the predominant internal corrosion mechanism as corrosive acid gases have largely been removed from these systems through processing and tank storage. Second, crude oil transmission pipelines are often operated under intermittent flow conditions where stagnant periods might allow for the accumulation of biogenic sulfide and the settling of sand and other particles.

Conclusions and outlook

The oil field microbiome is heavily affected by specialty chemicals which are widely used in the oil and gas industry for integrity management. The most prominent example in this context are biocides, which are injected for the very purpose of controlling microbial activity. Globally, 151 000 metric tons of organic biocides were used in 2017 to prevent or reduce the deleterious effects of environmental microorganisms in oil field operations (Beraud et al., 2018). On the other hand, CIs are injected in even larger volumes (404 000 metric tons in the same year), yet their role in microbial control and MIC has remained understudied and is often entirely overlooked in operational settings. In this study, we have shown that QAC-containing CIs can severely impede oil field biofilm formation and MIC under some conditions, while their use may lead to highly localized microbial corrosion in other instances where insufficient CI is available. CIs are pervasive in crude oil production, transportation and storage, and further research is needed to better understand and predict the influence of these integrity chemicals on the ecology and physiology of oil field microorganisms, particularly in the context of MIC.

Experimental procedures

Produced water sampling and laboratory incubation

Produced fluids (crude oil, water and associated gas) were collected in 5-gallon steel drums lined with an inert material at an oil-producing asset offshore Nigeria on 24 March 2016. Drums were capped to maintain anaerobic conditions and shipped to the United States at ambient temperature. In the laboratory, oily produced water (PW) was separated from crude oil through gravity separation and anaerobically transferred into 9 glass bottles (1 l, filled with 500 ml PW at pH 6.6) under a headspace of 21% (vol/vol) CO₂ and 79% (vol/vol) N₂ (CO₂:N₂) (Table S2). Each bottle was amended with an anaerobic sterile 1 M solution of Na₂SO₄ to reach a final concentration of 4.2 mM sulfate. In each microcosm, 3 carbon steel coupons (X52 grade carbon steel; exposed surface area = 1.25 cm² per coupon) were suspended vertically using an inert polyetheretherketone (PEEK) coupon holder, as described elsewhere (Fig. S1; Lahme et al., 2019). Sterilized sand (15 % wt/vol, Carbo Ceramics, Carbo Northern White™, 30/50 mesh) was added to 5 of the 9 microcosms. All microcosms were incubated for 15 months at 32°C on a clockwise-rotating shaker (75 r.p.m.) to allow for microbial growth and the study of MIC.

Laboratory incubations with synthetic produced water medium

To study the impact of CI under defined conditions, tests were set up with synthetic produced water medium, which was modelled after the West African field water chemistry (Table S2). The medium (1.6 l) contained (in g l⁻¹ water) NaCl (15.31), MgCl₂-6H₂O (0.562), CaCl₂-2H₂O (0.434), KCl (0.121), Na₂SO₄ (1.48), NaBr (0.064), NaCH₃CO₂ (0.996) and NaCl·3H₂O (0.157). Following transfer into 2 l glass bottles, the medium was purged with CO₂:N₂, sealed with a butyl rubber stopper and autoclaved. Once cooled, a sterile, anoxic stock solution (24.03 ml/1 l medium) of NaHCO₃ (1 M, equilibrated with pure CO₂) was added. A vitamin mixture,
trace elements, selenite–tungstate, phosphate, ammonium, vitamin B12, thiamine and riboflavin solutions (1 ml each per l of medium), prepared as described previously (Widdel and Bak, 1992), were added. Final pH of medium was between 6.5 and 6.8. One coupon (X52 grade carbon steel; 10 × 2.5 × 0.6 cm) was added to each bottle. This larger coupon size was used to better visualize macroscopic localized corrosion features surrounded by uncorroded metal surface. Five sides of each coupon had been sealed with a Teflon-based coating (Impreglon 800), so only one face of the coupon (2.5 × 10 cm) was exposed to the anoxic medium. Varying concentrations of a commercial CI formulation, ranging from 0 to 50 mg CI l⁻¹, were added. According to the safety data sheet (SDS) of the proprietary CI formulation, it contained 30–60% (wt/wt) methanol, 1–5% (wt/wt) isopropanol, 5–10% (wt/wt) organic sulfur-containing compounds and 16–45% (wt/wt) of a total of three different quaternary ammonium compounds (QACs). The microcosms were inoculated with 1% (vol/vol) of a produced water enrichment culture (please see Supporting Information) and incubated for 3 months at 32°C on a rotating shaker (75 r.p.m.).

CI residual measurement

CI in samples (10 ml) was measured using a Filming Amine (aliphatic amines) Test Kit (CHEMetrics, Midland, VA), according to the manufacturer’s instructions. Calibration curves for the colourimetric assays were generated using the proprietary CI formulation directly, or by using the model compounds benzylidimethyl-n-dodecylammonium chloride (C₁₂-QAC; Sigma-Aldrich) and benzylidimethyl-n-hexadecylammonium chloride (C₁₆-QAC; Sigma-Aldrich) dissolved in ultrapure water (18.2 MΩ cm). Absorbance of the chloroform-soluble colour complex was measured at 436 nm using a Thermo Scientific Genesys 10S UV-Vis spectrophotometer.

Dissolved sulfide measurements

Dissolved sulfide was determined as described elsewhere (Cord-Ruwisch, 1985) with some modifications. In brief, samples (500 µl) were filtered (0.2 µm) to remove precipitated particulate sulfides, added to 10 ml of an acidic copper sulfate solution (5 mM CuSO₄ in 50 mM HCl) and thoroughly mixed. The absorbance of colloidal CuS was measured immediately at 480 nm using a Thermo Scientific Genesys 10S UV-Vis spectrophotometer.

qPCR and 16S rRNA gene amplicon sequencing

Planktonic cells in culture medium or produced water (20 ml or 60 ml) were collected on Sterivex filters (0.2 µm) and stored at −80°C until analysis. Corrosion coupons were removed using sterile forceps and rinsed with sterile phosphate-buffered saline (PBS) solution. Biofilms were sampled using sterile swabs (minimum of 2 per coupon) and frozen at −80°C. For coupons with a surface area of 1.25 cm², the entire coupon was sampled, whereas for larger coupons, only ¼ of the surface area (6.25 cm²) was sampled. All frozen samples were shipped on ice to Microbial Insights (Knoxville, TN) for DNA extraction. DNA was extracted using the DNA Power Soil Total DNA Isolation kits (MO BIO Laboratories, Solana Beach, CA) according to manufacturer’s instructions.

Quantification of total eubacteria was performed on a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Grand Island, NY) using PCR primers and TaqMan probes targeting highly conserved regions (V9) of bacterial 16S rRNA genes based on primers described previously (Suzuki et al., 2000). Primers to enumerate archaeal 16S rRNA genes were developed by Microbial Insights based on previously published primer sets targeting the V5-V9 regions (Yu et al., 2005). All qPCR experiments included appropriate negative (no DNA) and positive control reactions. No amplification was detected in negative controls (CT > total cycles).

16S rDNA libraries were generated by GENEWIZ (South Plainfield, NJ) using its proprietary 16S MetaVx™ amplification primers, PCR conditions and other library preparation protocols. Briefly, DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and DNA quality was assessed on a 0.6% agarose gel. Sequencing libraries were constructed using a MetaVx™ 16S rDNA Library Preparation kit (GENEWIZ). Generated amplicons covered the V3–V5 hypervariable regions of the 16S rRNA gene. Indexed adapters were added to the ends of these amplicons by limited cycle PCR. Sequencing libraries were validated using a DNA chip for the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and quantified with the Qubit Fluorometer and real-time PCR (Applied Biosystems, Carlsbad, CA). DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to manufacturer’s instructions (Illumina, San Diego, CA). Sequencing was performed using a 2 × 250 paired-end configuration. Image analysis and base calling were conducted by the MiSeq Control Software on the MiSeq instrument. Initial taxonomy analysis was carried out on Illumina Basespace cloud computing platform.

The QIIME data analysis package was used for 16S rRNA data analysis (Caporaso et al., 2010). Forward and reverse reads were joined and assigned to samples based on barcodes and then truncated by cutting off the barcode and primer sequence. Quality filtering on joined sequences was performed, and sequences that did not
fulfil the following criteria were discarded: sequence length < 200 bp, no ambiguous bases, mean quality score < 20. Sequences were compared with the Ribosomal Database Program (RDP) Gold Database (Cole et al., 2014), using the UCHIME algorithm (https://drive5.com/uchime/uchime_download.html) to detect chimeric sequences. Once chimeric sequences were removed, the remaining sequences, ranging from 29 424 to 1 331 654 reads, were subjected to subsequent analyses. Sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH (1.9.6), using the Silva 119 database (Quast et al., 2012) pre-clustered at 97% sequence identity. The RDP classifier was used to assign taxonomic categories to all OTUs at confidence thresholds of 0.8. The RDP classifier uses the Silva 119 database which has taxonomic categories predicted to the species level. To evaluate how well each of these OTUs cluster, JMP Pro 14 was used to perform a principal components analysis using pairwise estimation of proportion normalized reads.

Data availability
The 16S rRNA gene amplicon sequencing data have been submitted to the NCBI short read archive (SRA) under the BioProject PRJNA606091.

Weight loss corrosion rate determination
Carbon steel coupons were sequentially sonicated in hexane, acetone and methanol (5 min each), dried in a stream of air and placed under vacuum overnight. Coupons were weighed thrice using an analytical balance, sterilized in ethanol (10 min for biotic tests; 30 min for abiotic tests) and dried under a stream of filtered N₂ gas prior to addition to the microcosms.

Following incubation, coupons were cleaned of corrosion products and other deposits in a warm (70°C) HCl solution (1.8 N) containing 2% propargyl alcohol (one min), neutralized in a saturated calcium hydroxide solution (30 s), scrubbed using a non-scratching nylon brush and rinsed in deionized water (Enning et al., 2016). This cleaning process was repeated before coupons were rinsed in acetone and dried in a stream of air. Coupons were placed under vacuum overnight to ensure complete drying, weighed three times using an analytical balance, and the weight loss (Table S1) was converted into a general corrosion rate normalized to the exposed coupon surface and assuming a steel density of 7.87 g cm⁻³.

Localized corrosion analyses
The surface topography of cleaned coupons was mapped using a Keyence VR-3000K Wide-Area 3D profile measuring macroscope. Localized corrosion features (≥ 25 μm deep) were quantified by comparison with an external standard and the deepest feature, with reference to the original (pre-corrosion) z-plane for each coupon was converted into an annualized pitting corrosion rate through linear extra- or interpolation.

Biofilm staining and imaging
Corrosion coupons covered with biofilm were rinsed with sterile PBS to remove planktonic cells and were then transferred to sterile glass petri dishes. A working solution of fluorescent dyes was made by mixing SYTO9® and propidium iodine (LIVE/DEAD™ BacLight™ Bacterial Viability Kit, ThermoFisher Scientific) in ⅓ strength PBS. This dye solution was added to ⅓ of the coupon surface area (6.25 cm²) and incubated at room temperature for 20 min in the dark. Subsequently, the glass petri dish was filled with ⅓ strength PBS until the liquid level was 1 cm above the surface of the coupon. The biofilm on the coupon was imaged using a Zeiss Axio Imager.Z2m Live Cell Instrument MagLevit™ with water lenses and equipped with an X-cite series 120 Q lamp (Lumen Dynamics).

Acknowledgements
We would like to thank Ramsey J. Smith and John Longwell for technical assistance as well as Fang Cao, Yao Xiong, David Fischer and Conchita Mendez for valuable discussion.

Conflict of interest
The authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supplementary methods.

Fig. S1. Modified 1 l Duran bottle for the study of microbially influenced corrosion (MIC). Bottles are sealed with a gas-tight butyl rubber septum and contain a coupon holder made of electrically insulating polyether ether ketone (PEEK) that houses three carbon steel coupons. The coupon holder is inserted into anaerobic produced water (PW).

Fig. S2. Scavenging of corrosion inhibitor (CI) with laboratory-grade sand (SiO2). Sterilized sand (15% wt/wt) was added to glass bottles containing sterile anoxic produced water medium (n = 2). Following addition of 3 mg CI l⁻¹, CI concentration was monitored over 14 days. CI concentration in sterile control tests without sand (n = 2) is shown for comparison.

Fig. S3. Surface depth profiles of carbon steel coupons incubated in oily produced water for 15 months. Coupons incubated in sand-free bottles (n = 4) showed no signs of corrosion and remained virtually pristine, which was attributed to higher concentrations of residual CI. Addition of sand lowered CI concentrations in the other set of produced water incubations (n = 5) and highly localized corrosion,
Corrosion inhibitors impact microbial activity

Steel-attached biofilm and planktonic microbial communities in anoxic microcosms with synthetic produced water medium and carbon steel coupons. Microcosms (n = 3) were amended with 0 mg CI l⁻¹, 3 mg CI l⁻¹, 5 mg CI l⁻¹ or 10 mg CI l⁻¹ and incubated for 3 months. (A) Ses-sile bacterial and archaeal microbial community composition of biofilms collected from steel coupons. (B) Planktonic bac-
terial and archaeal microbial community composition in microcosm produced waters. (A) and (B) depict families which account for ≥ 2.5% of total reads (average of biological replicates) in at least one of the four data sets (0, 3, 5 or 10 mg CI l⁻¹).

Fig. S10. (A) Concentration of corrosion inhibitor (CI) in iron-free incubations containing synthetic produced water medium (SPWM). CI was added at 3 mg l⁻¹ to triplicate bottles containing either sulfide-free sterile SPWM (crosses), sterile SPWM with 0.8 mM Na₂S (diamonds), a heat-sterilized sulfidic culture (triangles), or an active sulfidic culture (circles). (B) Dissolved sulfide in incubations of SPWM.

Fig. S11. Microbially influenced decrease in corrosion inhibi-
tor (CI) concentration in aerobic artificial seawater medium (600 ml) with 0.5 g of coastal sediment. Inhibitor was added at 25 mg CI l⁻¹ to both, aerobic and anaerobic cultures. Sterile controls were obtained through heat-sterilization of medium containing sediment, prior to addition of CI.

Fig. S12. Concentration of the quaternary ammonium com-
pounds (QAC) benzyltrimethyl-n-dodecylammonium chloride (a C12-QAC) and benzyltrimethyl-n-hexadecylammonium chloride (a C16-QAC) in synthetic produced water medium containing oil field microorganisms and carbon steel cou-
pons (A and B). QAC concentrations in abiotic controls are also shown. (C) Surface depth profile of carbon steel cou-
pons exposed to produced water medium for 2 months. Scale bar = 2.5 cm, white arrow and dashed circle denote localized corrosion features.

Table S1. Raw data used to calculate weight loss corrosion rates.

Table S2. Chemical composition and qPCR assay results of the West African produced water.