Preliminary Investigation of Utilization of a Cellulose-Based Polymer in Enhanced Oil Recovery by Oilfield Anaerobic Microbes and its Impact on Carbon Steel Corrosion

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Water injection increases reservoir pressure in enhanced oil recovery (EOR). Among other oilfield performance chemicals, an EOR polymer is added to the injection water to provide the viscosity necessary for effective displacement of viscous crude oil from the reservoir formation. However, these organic macromolecules may be degraded by microbes downhole, causing undesirable viscosity loss. The organic carbon utilization by the microbes promotes microbial metabolism, thus potentially exacerbating microbiologically influenced corrosion (MIC). In this preliminary laboratory investigation, 3,000 ppm (w/w) carboxymethyl cellulose sodium (CMCS), a commonly used EOR polymer, was found to be utilized by an oilfield biofilm consortium. This oilfield biofilm consortium consisted of bacteria (including that can degrade large organic molecules), sulfate-reducing bacteria (SRB), and other microorganisms. A 30-day incubation in 125 mL anaerobic vials was conducted with an artificial seawater medium without yeast extract and lactate supplements at 37°C. The polymer biodegradation led to 16% viscosity loss in the broth and a 30× higher SRB sessile cell count. Slightly increased MIC weight loss and pitting corrosion were observed on C1018 carbon steel coupons. Thus, the use of CMCS in EOR should take into the consideration of microbial degradation and its impact on MIC.

KEY WORDS: biodegradation, biofilm, carboxymethyl cellulose sodium, enhanced oil recovery, microbiologically influenced corrosion, viscosity

INTRODUCTION

Aging reservoirs require enhanced oil recovery (EOR) to sustain oil production.[1] For offshore and near-shore operations, seawater is used in flooding together with EOR chemicals (e.g., polymers) to counter depleting reservoir pressures.[2] However, the practice can promote microbial growth.[3] Sulfate-reducing bacteria (SRB) can flourish in the anaerobic downhole environment.[4] Some sessile SRB cells can utilize the electrons from elemental iron for sulfate respiration via direct or indirect extracellular electron transfer.[1] Biogenic H_2S produced by SRB can lead to reservoir souring and also stress corrosion cracking (SCC) under certain conditions.[5]

Environmental microbes live in synergistic biofilm consortia in the field environments.[6-10] It is generally true microbiologically influenced corrosion (MIC) are caused by biofilms.[11-20] On the other hand, a biofilm can also prevent corrosion, for example, by forming a mass transfer barrier to block a corrosive agent such as O_2.[21-22] SRB are frequently found to be responsible for MIC because sulfate is widely available in anoxic and anaerobic environments.[23-25] Acid-producing bacteria (APB) can also cause MIC if they generate a sufficiently acidic local pH underneath APB biofilms. Some researchers also pointed out the role of methanogens in MIC.[26-27] Because EOR polymers are typically organic macromolecules, it is necessary to investigate the biodegradability of some EOR polymers by oilfield microbes, which can potentially promote microbial growth and MIC.

Polymer addition increases the viscosity of the injected fluid so that viscous crude oil can be displaced from rock formations in reservoirs.[28] In EOR, polymers such as cellulose-based polymers, partially hydrolyzed polyacrylamide (HPAM),[29] and xanthan gum are common choices.[30] Xanthan gum, which is used sometimes in food preparations, is readily biodegradable, resulting in viscosity loss.[31] HPAM is more commonly used nowadays.[32] It was found to be utilized by an SRB which led to viscosity loss.[33] Jia, et al., reported that a commercial HPAM product for EOR was utilized by an oilfield biofilm consortium, causing increased carbon steel corrosion in addition to viscosity loss.[34] *Bacillus* spp. are known to degrade HPAM to utilize it as carbon and nitrogen sources.[35]

Cellulose-based EOR polymers have become popular in recent years.[36] However, microbes such as *Bacillus licheniformis* can produce cellulase enzymes for cellulose degradation.[37] Although there was no report confirming direct degradation of cellulose-based polymers used for EOR by SRB, SRB are known to utilize fermentation products of cellulose (e.g., organic acids) as organic carbons to promote their growth.[38-39] In nature, microbes usually live in a synergistic community which can include fermentative microbes that are able to degrade cellulose-based polymers to provide short-chain organic nutrients to SRB.

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In this work, a commercial cellulose-based polymer used in EOR operations was added to an artificial seawater culture medium incubated for 30 d to investigate its biodegradation by an oilfield biofilm consortium. The impact of the biodegradation on MIC was assessed.

MATERIALS AND METHODS

2.1 | Coupon, Biofilm, and Culture Medium Composition

Square C1018 (UNS G10180[(1)]) carbon steel coupons (10 mm × 10 mm × 5 mm) were coated with inert polytetrafluoroethylene except for the top 1 cm^2 test surface. The test surface was abraded sequentially with 180, 400, and 600 grit abrasive papers. The coupons were degreased with anhydrous isopropanol before being dried under UV light in an anaerobic chamber. An oilfield mixed-culture biofilm (codenamed biofilm "Consortium II") was used. Its constituents included SRB, microbes that degrade recalcitrant organic molecules, and fermentative microbes. The microbial community composition of Consortium II was previously published.[40] An artificial seawater culture medium was used in this work. Its composition (g/L) was: NaCl 23.476, $Fe(NH_4)_2(SO_4)_2$ 0.500, Na_2SO_4 3.917, $CaCl_2{\cdot}2H_2O$ 1.469, $NaHCO_3$ 0.192, NH_4Cl 0.100, KCl 0.664, $CaSO_4{\cdot}2H_2O$ 0.100, KBr 0.096, $MgSO_4{\cdot}H_2O$ 0.400, H_3BO_3 0.026, tri-sodium citrate 0.500, $MgCl_2{\cdot}6H_2O$ 10.610, K_2HPO_4 0.050, $SrCl_2{\cdot}6H_2O$ 0.040. Citrate served as a chelator to protect Fe^{2+} from excessive precipitation due to iron sulfide formation so Fe^{2+} is available as an enzyme co-factor for microbes such as SRB.[41-45] The artificial seawater medium's pH was adjusted to 7.5 by using an HCl solution. The artificial seawater medium was enriched with sodium lactate (3.5 g/L) and yeast extract (1 g/L) to grow the Consortium II seed culture. However, the artificial seawater was not enriched with the yeast extract and lactate for testing the biodegradation of the EOR polymer. Commercial carboxymethyl cellulose sodium (CMCS) was provided by Petronas of Malaysia.

2.2 | Effect of CMCS Biodegradation on Microbial Growth

The artificial seawater culture medium was used for the CMCS-free incubation of Consortium II. In one set of the artificial seawater medium, 3,000 ppm (field operation concentration) CMCS was added and stirred at 400 rpm for more than 6 h to fully dissolve CMCS in the culture medium. The abiotic control contained 3,000 ppm CMCS in the artificial seawater medium without inoculation. All of the culture media were autoclaved at 121°C for 20 min. All liquid solutions for anaerobic incubation were deoxygenated with filtered N_2 for 1 h to assure <40 ppb (w/w) dissolved oxygen to diminish oxygen corrosion effect. A filter-sterilized L-cysteine stock solution was added to reach 100 ppm (w/w). It served as an O_2 scavenger to reduce dissolved oxygen further and to deal with possible O_2 leaks. Each 125 mL anaerobic vial (Wheaton Industries Inc., Millville, NJ, USA) had 100 mL culture medium and 5 replicate C1018 coupons. One mL Consortium II seed culture inoculated each biotic vial (initial planktonic cell count 10^6 cells/mL after inoculation) in an anaerobic chamber. After that, all vials were sealed and then incubated for 30 d at 37°C statically. The incubation process was repeated at least three separate times. The viscosity of the culture media containing 3,000 ppm CMCS with and without inoculation was monitored during the 30-d incubation using a falling ball viscometer at room temperature (23°C).[46] In each separate experiment, at least 10 bottles (5 for abiotic and 5 for biotic) were incubated to collect viscosity data. The t-test method was used to calculate all p-values.

One set of inoculated vials were used for planktonic cell counting using a hemocytometer during the incubation. In each separate experiment, at least 32 bottles (16 for 0 ppm CMCS and 16 for 3,000 ppm CMCS) were incubated to collect planktonic cell count data. A syringe with a needle was used to withdraw a 0.3 mL broth sample periodically to count planktonic cells. On days 7, 14, 21, and 30, sessile SRB cell counts on the coupons were checked with most probable number (MPN) method. In each separate experiment, at least 8 bottles (4 for 0 ppm CMCS and 4 for 3,000 ppm CMCS) were incubated to collect sessile SRB cell count data. A modified Postgate's B liquid medium for SRB from Biotechnology Solutions (Houston, TX, USA) was used for MPN. Each sessile cell count was based on three replicate rows (series) of 10 mL serum vials. Before the sessile cells were counted, the coupons retrieved from anaerobic vials were rinsed with pH 7.4 PBS (phosphate buffered saline) solution in a biosafety cabinet at 23°C. Then, the biofilm and corrosion products were scratched off from each coupon surface with a sterile applicator into a 10 mL pH 7.4 PBS solution. The cell suspension was shaken to distribute cells evenly before the sessile cell enumeration.[7] During the process, sessile cells were exposed to oxygen only briefly prior to enumeration. This would not affect the observed relative trends in sessile cell viability.

After the 30-d incubation, biofilms were examined using a scanning electron microscopy (SEM) machine (JEOL JSM-6390[†], Tokyo, Japan). The procedure to prepare coupons for SEM examination was reported before.[47] Live and dead sessile cells were observed with a confocal laser scanning microscope (CLSM) (Carl Zeiss LSM 510[†], Jena, Germany) as reported before.[7] Live/Dead® BacLight™ Bacterial Viability Kit L7012[†] (Life Technologies, Grand Island, NY, USA) consisting of a green-fluorescent stain and a red-fluorescent stain was usedto stain biofilms before CLSM observations. Under CLSM, dead cells show up as red dots and live cells green. In each separate experiment, at least 4 bottles (2 for 0 ppm CMCS and 2 for 3,000 ppm CMCS) were incubated to collect SEM and CLSM data.

2.3 | Corrosion Analyses

After 30 d of incubation, coupons were retrieved. Each coupon's surface was cleaned with a freshly prepared Clarke's solution before weighing.[48] The same coupon was used to inspect pit morphology under SEM. Pit profiles on the coupons were obtained using a profilometer (Alicona Imaging GmbH ALC13[†], Graz, Austria). In each separate experiment, at least 4 bottles (2 for 0 ppm CMCS and 2 for 3,000 ppm CMCS) were incubated to collect weight loss and pitting data.

RESULTS

Figure 1 shows that the viscosity of the abiotic artificial seawater medium containing 3,000 ppm CMCS was almost unchanged during the 30-d incubation at about 6.4 cp. In the inoculated medium with CMCS, the viscosity continuously decreased. At the end of the 30-d incubation, the viscosity had a 16% decrease.

(1) UNS numbers are listed in *Metals & Alloys in the Unified Numbering System*, published by the Society of Automotive Engineers (SAE International) and cosponsored by ASTM International.

† Trade name.

Figure 2 shows that in the inoculated medium without CMCS, the planktonic cell count had a continuous decline during the entire incubation period. With 3,000 ppm CMCS, the planktonic cell count decreased initially in the first 2 d and then leveled off before increasing again on day 6. On the 10th day, it started to decline slowly. At the end of the 30-d incubation, 3,000 ppm CMCS led to a higher planktonic cell count (2×10^5 cells/mL) than that without CMCS (2×10^4 cells/mL). Figure 3 demonstrates that without CMCS, the sessile SRB cell count (cells/cm^2) declined by 30 times during the 30-d incubation, while with CMCS utilization, it did not decline. Sessile SRB cell counts were important because SRB were the main corrosive species in Consortium II in the non-acidic broth.[40]

After the 30-d incubation, biofilm morphologies were observed under SEM. Different cell shapes are seen in Figures 4(a) (without CMCS) and (b) (with 3,000 ppm CMCS). Generally speaking, more sessile cells and extracellular polymeric substance are seen in Figure 4(b) than those in (a). CLSM can detect live and dead sessile cells in a biofilm. Figure 4(a') shows that the sessile cells incubated without CMCS appear mostly dead (red dots), while those incubated with CMCS appear live (green dots) (Figure 4[b']). Without CMCS, the biofilm was thinner than that with CMCS (Figure 4).

The (specific) weight losses of the abiotic coupon incubated with CMCS, the biotic coupon incubated without CMCS, and the biotic coupon incubated with CMCS were 0.3±0.1 mg/cm^2, 3.1±0.3 mg/cm^2, and 3.8±0.3 mg/cm^2, respectively, after the 30-d incubation (Figure 5). The calculated uniform corrosion rates based on the average weight loss values were 0.048 mm/y and 0.059 mm/y for the biotic coupon incubated without CMCS and the biotic coupon incubated with CMCS, respectively. In Figure 5, the biotic coupon incubated with 3,000 ppm CMCS had a slightly higher (p-value = 0.04 < 0.05) weight loss than that without CMCS. The pH values of the abiotic medium containing 3,000 ppm CMCS, the biotic medium with 0 ppm CMCS, and the biotic medium with 3,000 ppm CMCS after the 30 d of incubation were 7.5±0.1, 7.7±0.2, and 7.5±0.2, respectively, not deviating much from the initial pH of 7.5.

Figure 6 exhibits corrosion pits on the abiotic coupon incubated with 3,000 ppm CMCS, on the biotic coupon incubated without CMCS, and on the biotic coupon incubated with 3,000 ppm CMCS after the 30-d incubation. Negligible pitting corrosion with well-preserved polishing lines is seen on coupons exposed to the abiotic medium containing CMCS in Figures 6(a) and (a'). Many corrosion pits are seen on the coupons exposed to the biotic medium without CMCS in Figures 6(b) and (b'). More aggressive pitting can be seen on the biotic coupons exposed to CMCS in Figures 6(c) and (c'). The average maximum pit depth data were calculated from six samples which came from three separate experiments. The average maximum pit depths of the biotic coupons incubated without CMCS, and the biotic coupons incubated with CMCS were 22.7±2.8 μm and 30.8±3.1 μm, respectively, after the 30-d incubation, as shown in Figure 7. The biotic coupon incubated with 3,000 ppm CMCS had a higher (p-value = 0.03 < 0.05) pit depth than that without CMCS. Based on the concept of RPS (relative pitting severity) introduced in Equation (1),[49]

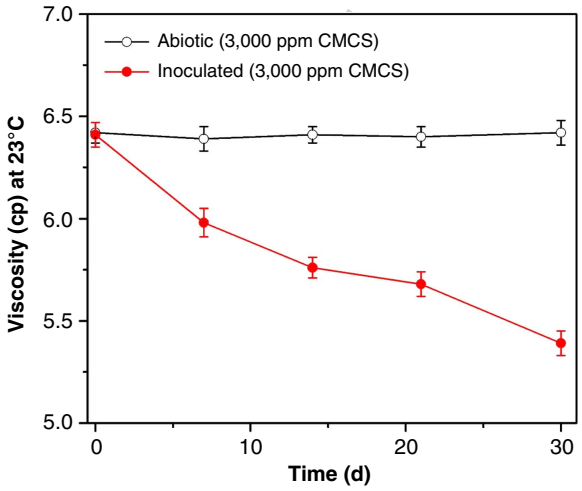

FIGURE 1. *Viscosities of artificial seawater medium containing 3,000 ppm CMCS with and without inoculation during a 30-d incubation period at 37°C. Error bars represent standard deviations from three separate experiments.*

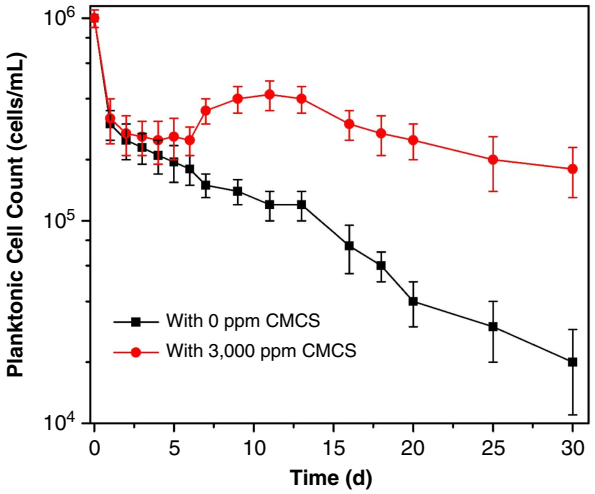

FIGURE 2. *Planktonic cell counts in inoculated artificial seawater medium with and without 3,000 ppm CMCS. Error bars represent standard deviations from four separate experiments.*

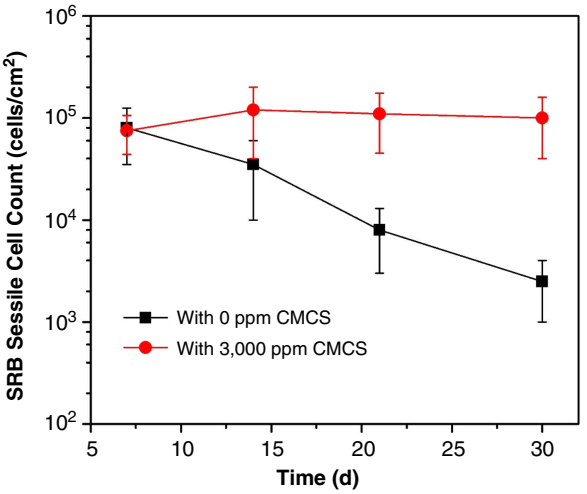

FIGURE 3. *Sessile SRB cell counts in inoculated artificial seawater medium with and without 3,000 ppm CMCS. Error bars represent standard deviations from four separate experiments.*

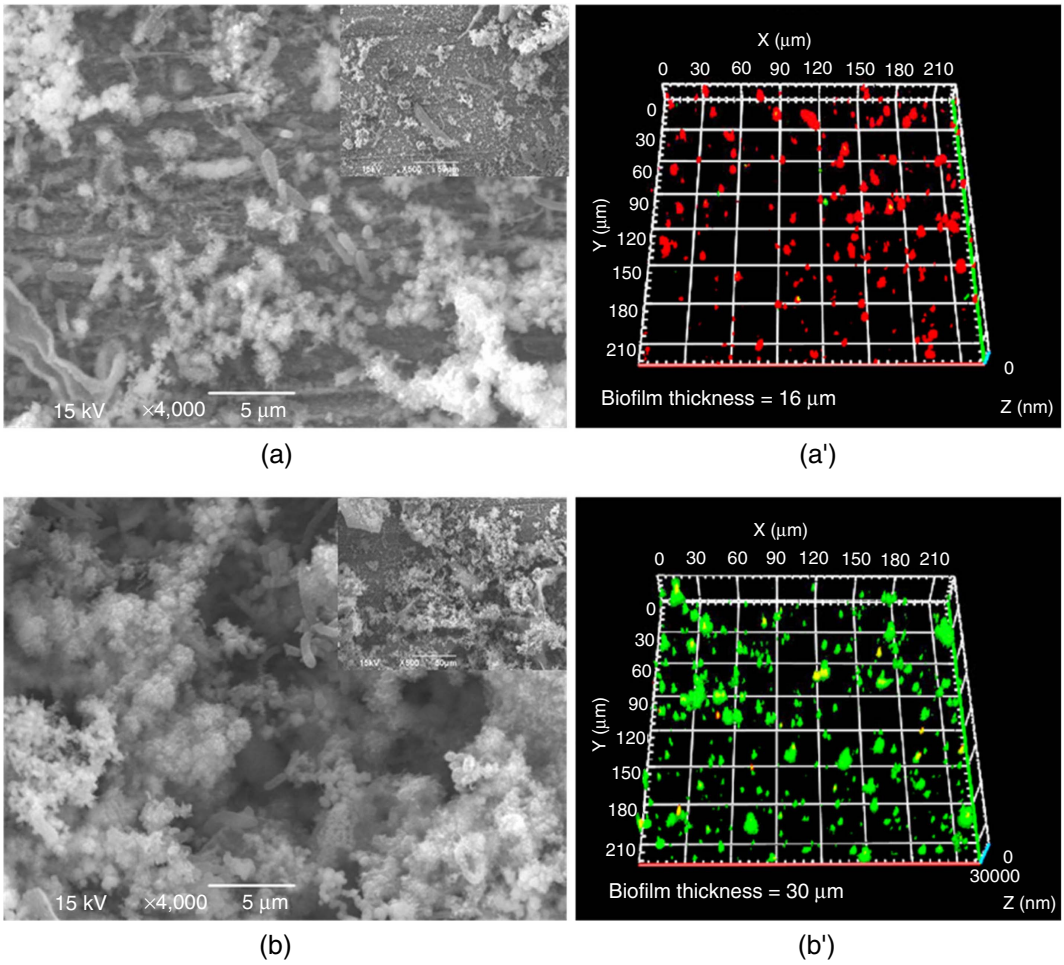

FIGURE 4. *Biofilm SEM and CLSM images on the coupon surfaces after the 30-d incubation: (a, a') in inoculated artificial seawater medium with 0 ppm CMCS, and (b, b') in inoculated artificial seawater medium with 3,000 ppm CMCS. Red dots indicate dead cells while green dots indicate live cells.*

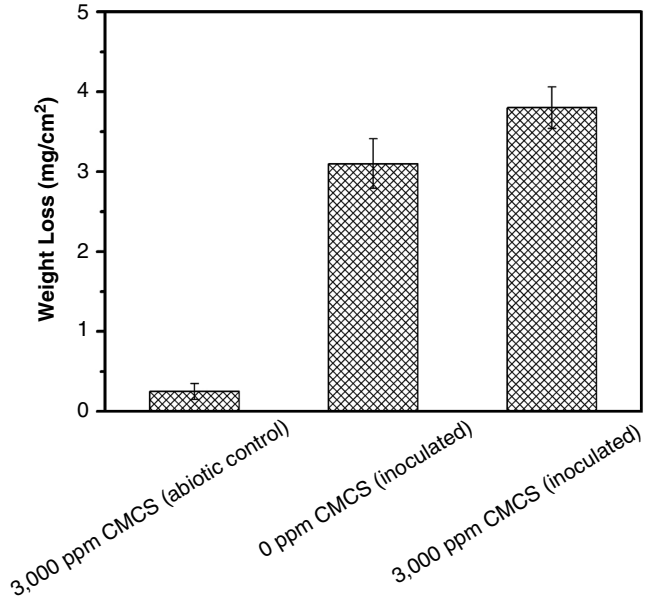

FIGURE 5. *Weight loss data of coupons after the 30-d incubation in abiotic artificial seawater medium with 3,000 ppm CMCS and inoculated artificial seawater medium with and without 3,000 ppm CMCS. Error bars represent standard deviations from six coupons gathered evenly from two separate experiments.*

$$\text{RPS} = \frac{\text{maximum pit growth rate}}{\text{uniform corrosion rate based on (specific) weight loss}} = \frac{\text{maximum pit depth} \times \text{metal density}}{\text{(specific) weight loss}} \quad (1)$$

the two biotic pit depth values and the corresponding biotic weight losses above yielded RPS values of 5.7 and 6.3 for the biotic coupons incubated without CMCS and with CMCS, respectively. They were both much larger than unity, indicating that pitting corrosion was far more important than uniform corrosion in this work.

DISCUSSION

Viscosity results in Figure 1 clearly indicate that CMCS was degraded by biofilm Consortium II. The planktonic cell count decrease in the inoculated medium without CMCS was the result of depleted organic carbon to support cell growth. Figure 2 shows that with CMCS, Consortium II adapted to using this new organic carbon and this stopped the decline of the planktonic cell count after 5 d of incubation. The sessile SRB cell growth also benefited from the help of CMCS. It was likely that SRB benefited from carboxymethyl cellulose degradant products or from metabolites produced by other microbes that

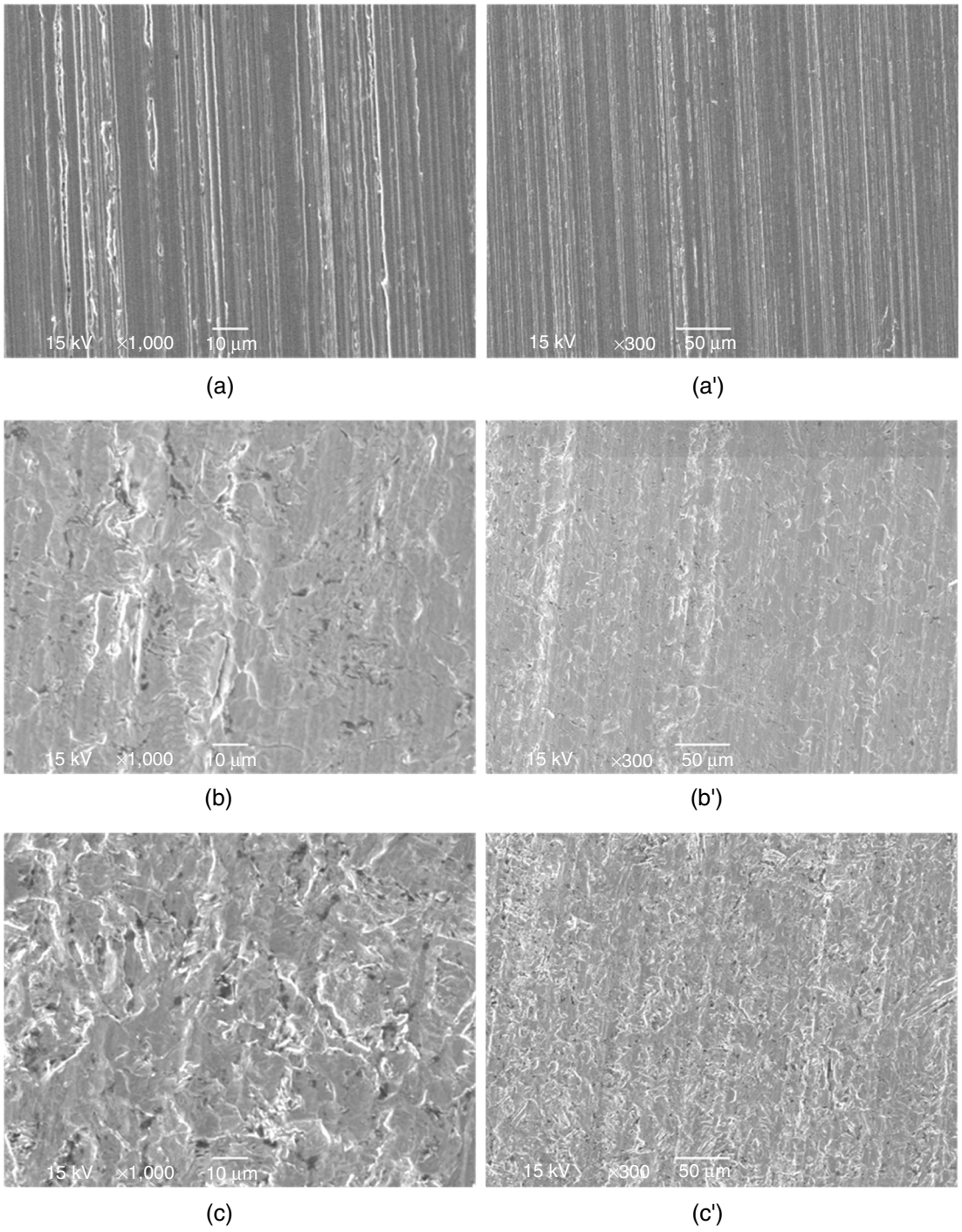

FIGURE 6. *Pit SEM images (apostrophe indicates a smaller magnification) of coupons after 30-d incubation with biofilms and corrosion products removed: (a, a') in abiotic artificial seawater medium with 3,000 ppm CMCS, (b, b') in inoculated artificial seawater medium with 0 ppm CMCS, and (c, c') in inoculated artificial seawater medium with 3,000 ppm CMCS.*

degraded cellulose.[39] The biofilm images in Figure 4 generally corroborate sessile SRB cell count data in Figure 3. The negligible coupon weight loss for the abiotic control indicating the corrosion effect of the chemicals in the abiotic vials including artificial seawater ingredients and CMCS in an anaerobic system was negligible. The SEM pit images (Figure 6) corroborate the weight loss data trend (Figure 5). The pit depth data (Figure 7) are consistent with SEM pit images and weight loss data.

Generally speaking, there are two main mechanisms for MIC caused by microbes under anaerobic condition. They do not include MIC by a pre-existing corrosive agent (e.g., CO_2) that is accelerated by the microbial damage of passivation films. The first type of MIC is known as extracellular electron transfer MIC

(EET-MIC).[50-54] In this type of MIC, electrons from metals such as elemental iron with a relatively low reduction potential for Fe^{2+} are used by sessile cells to reduce a non-oxygen electron acceptor such as sulfate in a cell's cytoplasm. The reduction reaction needs biocatalysis in the cytoplasm. However, iron oxidation happens outside the cell because the metal has no solubility in water. Therefore, EET is necessary to bridge the oxidation and reduction reactions. The following two reactions explained the SRB MIC using extracellular electrons from iron oxidation with sulfate as the terminal electron acceptor.[55]

$$Fe \rightarrow Fe^{2+} + 2e^{-} \quad (2)$$

$$SO_4^{2-} + 9H^{+} + 8e^{-} \rightarrow HS^{-} + 4H_2O \quad (3)$$

In bioenergetics, the redox reaction combining the two half reactions above is thermodynamically favorable (at 25°C, pH 7 and 1 M solutes/1 bar gases) with energy release.[56] In fact, iron granules have been used in evolutionary microbiology research to serve as the sole energy source (i.e., sole electron donor) for SRB growth using sulfate as the terminal electron acceptor.[55,57]

Despite the favorable thermodynamics for SRB MIC of Fe^0, the actual corrosion rate is dictated by corrosion kinetics. This explains why the coupon weight loss was negligible in the abiotic medium although both iron and sulfate were present. In the biotic media, moderate corrosion is seen in Figures 5 through 7.[58] This was because sulfate reduction under biocatalysis was performed by SRB cells. The corrosion was caused by sessile cells instead of planktonic cells because planktonic cells in the bulk fluid could not perform EET between cells and the metal surface due to the presence of the bulk fluid. However, in this work planktonic cell counts were also important in the overall picture, because both planktonic cells and sessile cells degraded CMCS.

The CMCS addition to the artificial seawater (not supplemented with yeast extract and lactate) arrested the decline of planktonic and sessile SRB cell counts (Figures 2 and 3). CMCS as evidenced by the continuous viscosity decline of the broth is seen in Figure 1. The enhanced SRB growth is indicated by the darkened broth color in Figure 8. More FeS precipitation (black color) is seen in the biotic medium with 3,000 ppm CMCS compared with that in the biotic medium without CMCS. This was due to the fact that more SRB cells produced more HS^- and thus precipitated more Fe^{2+} to form FeS in Reaction (4).

$$Fe^{2+} + HS^{-} \rightarrow FeS + H^{+} \quad (4)$$

Metabolite MIC (M-MIC) is another type of anaerobic MIC (i.e., MIC caused by anaerobes). It is caused by corrosive metabolites (oxidants) with the notable example of organic acids secreted by APB.[51,59] The secreted organic acids underneath an APB biofilm can generate a locally acidic condition underneath the film. In this work, the culture medium pH values in both the abiotic and biotic vials with and without CMCS were over 7.5. This non-acidic broth pH indicates that M-MIC due to acid producers or H_2S could not be a major contributor to MIC. The clear association of more sessile SRB cells (Figure 3) with more severe MIC (Figure 6) due to the presence of CMCS in the inoculated artificial seawater suggested that EET-MIC by SRB was the main corrosion mechanism in carbon steel MIC by SRB with non-acidic broth pH. In addition to SRB, which dominated in the consortium, other electroactive organisms in the consortium might also contribute to EET-MIC.

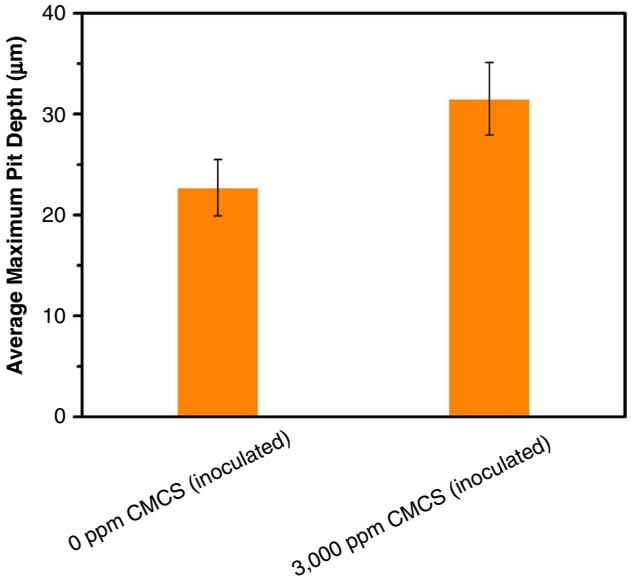

FIGURE 7. *Average maximum pit depth of coupons after the 30-d incubation in inoculated artificial seawater medium with and without 3,000 ppm CMCS. Error bars represent standard deviations from six coupons gathered from three separate experiments.*

It is interesting to note that although weight loss and pit depth increased considerably in the presence of CMCS, RPS remained around 6.0. This is not too far from the RPS value of 6.8 reported by Dou, et al.,[49] for C1018 corrosion by *Desulfovibrio vulgaris* in ATCC 1249 medium. An RPS value much larger than unity means pitting is far more important than general corrosion (uniform corrosion).

(a) (b)

FIGURE 8. *Anaerobic vials inoculated with biofilm Consortium II after 30-d incubation in artificial seawater medium: (a) with 0 ppm CMCS, and (b) with 3,000 ppm CMCS.*

CONCLUSIONS

➢ Experimental data in this work demonstrated that the cellulose-based polymer CMCS was degraded by an oilfield mixed-culture biofilm in anaerobic vials filled with an artificial seawater medium. Due to the CMCS degradation, the viscosity of the biotic medium with 3,000 ppm CMCS decreased by 16% after 30 d of incubation at 37°C. The utilization of CMCS promoted the growth of planktonic cells and sessile SRB cells. This led to more severe pitting corrosion on carbon steel coupons compared with the coupons incubated without CMCS. The results in this work will help oil and gas industry operators select a suitable EOR polymer and assess the need for biocide dosing during EOR.

NOTES

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