The effect of *Acetobacter* sp. and a sulfate-reducing bacterial consortium from ethanol fuel environments on fatigue crack propagation in pipeline and storage tank steels


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**Abstract**

This paper evaluates the effects of microbiologically influenced corrosion (MIC) on fatigue-crack growth of candidate materials useful in expanding bio-ethanol usage, including a storage-tank steel (ASTM A36) and two pipeline steels (API 5L X52 and X70). The microbiological species sampled and cultivated from an ethanol fuel production stream are responsible for both acetic acid and hydrogen sulfide production that lead to significant increases in fatigue-crack growth rate across a wide range of stress-intensity-factor amplitudes (AK). The mechanism for increased fatigue damage is hydrogen uptake through adsorption into the steel, which embrittles material ahead of the growing fatigue crack.

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1. Introduction

Understanding the corrosive effects of alternative fuel environments on fuel storage and transport infrastructure is important for predicting mechanical integrity of various components of the fuel delivery infrastructure. At a minimum, these components include pipelines, valves, storage tanks, ships, trucks and railcars. The existing infrastructure in the U.S., and throughout many other countries, was designed to handle fossil fuels but could be repurposed to handle emerging alternative fuels. A major concern is that the corrosive effects of alternative fuels are not well known for existing transport and storage scenarios.

Fuel-grade ethanol (FGE) is one particular alternative that is seeing significant increases in consumption rates throughout much of the world where biomass that is conducive for ethanol fuel production is available. FGE is a desirable alternative to fossil fuels since it can be blended with gasoline fuels already in use, as well as serve as an oxygenator to reduce particulate emissions during the combustion process [1]. However, the sources of biomass for FGE are typically located in rural areas where the fuel is produced. The fuel then needs to be transported long distances into existing infrastructure by railroad, truck, and barge tankers to primarily coastal regions where consumption rates are very high [2]. Pipelines offer significant safety advantages and improved cost-effectiveness compared to other shipping methods if the corrosive effects are predictable, especially with respect to their mechanical integrity. However, degradation of the mechanical integrity of ethanol piping and steel tanks is established due to the well-known phenomenon of ethanol stress corrosion cracking [3–6].

Microbiologically influenced corrosion (MIC) can be a significant problem in the material systems that handle, store, transport, and distribute fuels [7–15]. The onset of MIC in pipeline systems is a result of many causes, from inadequate water handling practices during hydrostatic testing of pipelines to lack of water chemistry control, lack of biocide usage and oxygen scavenging, all of which can lead to the premature degradation of alloys [27]. Aside from pipelines, fuel storage tanks are also susceptible to MIC, which can take the form of general corrosion and pitting, when exposed to a variety of fuel systems [11–13,18,20]. Biocides are frequently applied to storage tank systems as part of an anti-microbial strategy, but both the presence of microbial species and microbial biocide resistance may vary with the fuel source. Sulfate-reducing bacteria (SRB) and acid-producing bacteria (APB) in particular have both been known to contribute to the MIC of engineered alloys including steels [7].

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SRB species have been the focus of many corrosion studies and are known to cause corrosion in pipeline systems associated with fossil fuels and be particularly problematic due to the production of corrosive hydrogen sulfide [16,19,23,26,28,29]. The presence of molecular hydrogen (H₂), and SRB-produced hydrogen sulfides enhance anodic dissolution, and have an embrittling effect on steels. The hydrogen sulfides in particular slow the recombination of molecular hydrogen so that its uptake into the steel has more time to proceed. In contrast, APB induce corrosion by metabolism of an organic substance, such as a carbon source and H₂ as an electron donor resulting in secretion of corrosive organic acids [7]. Studies have shown that APB influence corrosion of pipeline steels [19] and that microbial production of acetic acid enhances corrosion [30]. Pope et al. show that the observed corrosion damage by APB was distinct from corrosion produced by protic acid of the same pH, including that produced by SRB [30]. Though SRB and APB have been implicated in corrosion of steels, often, different types of microbes with various metabolic capabilities inhabit steel surfaces as biofilms and impact corrosion processes. Microbially films, which are communities of microbes immobilized by an organically produced extracellular polymeric substance (EPS), can influence corrosion processes because they produce environments at the metal/biofilm interface that can have low values of pH and dissolved oxygen, and since they produce electrochemical reactions that significantly differ from those in the bulk solution [7].

Mechanical degradation in biologically active environments has been considered in several studies [26,31–38]. Pipelines and even fuel storage tanks (e.g., in railcars, barges, and tanker ships) undergo cyclic loading due to e.g., fluctuations of internal pressure and wave loading, respectively. The combination of pitting and cyclic loading can readily have the undesired effect of fatigue crack initiation [39]. Environmentally assisted fatigue–crack propagation and stress-corrosion cracking (SCC) become a concern since both lead to eventual mechanical degradation, reduced component life, or worse, failure and the associated environmental consequences. In regard to SRB in particular, fatigue–crack propagation rates in high-strength steels have increased by a factor of 50–1000 when freely corroding or when placed under applied cathodic potential [31,33,38]. These studies, which have focused on several offshore environments, have shown that the extensive damage accumulated in test materials was related to sulfide-enhanced hydrogen uptake. Mechanical testing in inoculated media can be problematic, since transient crack propagation behavior has been caused by increases in metabolically produced species, which can compromise structural life prediction with the fatigue data produced in the test [31].

Given that cyclic mechanical loading occurs in fuel transport systems and that microbial species sampled from a FGE production stream (including Desulfovibrio sp. and Acetobacter sp.) have reportedly influenced corrosion of structural and pipeline steels [40,41], further investigation is needed on the effect of microbial contamination of ethanol fuel environments on fatigue crack propagation. Specifically, the effect of the SRB Desulfovibrio sp. [42] and the APB Acetobacter sp. [43] on corrosion has been demonstrated in other systems and warrants further study. There is no description available of the effects that SRB and APB have on the cracking of steels used for ethanol pipelines and storage tank alloys under fatigue loading. This study evaluates the effect of these two types of microbes on the fatigue crack propagation in three steels that would be encountered in the storage and transport of alternative fuels such as fuel-grade ethanol. Our results may have more broad implications because similar species are found throughout the oil and gas industry.

2. Experimental procedures

2.1. Materials

Two grades of pipeline steel (API 5L X52 and X70) and a plate steel (ASTM A36) used for storage tank fabrication were obtained for this study from an oil & gas transmission company and a steel tank fabricator, respectively. The X52 pipe was 324 mm diameter with 9.53 mm wall thickness, and the X70 pipe was 508 mm diameter with 6.60 mm wall thickness. The A36 plate was 6.35 mm in thickness. Chemical compositions are shown in Table 1. Representative microstructures of the two pipeline materials are shown in Fig. 1. Metallographic specimens were sectioned from the pipeline materials and prepared with standard polishing methods for optical microscopy (1 mm final polish and 2% nital etch). The X36 and X52 steel alloys have similar microstructure and contain a mixture of polygonal ferrite and pearlite. The X70 material contains a fine-grained polygonal ferrite and bainitic structure. Note that the finer grain size in the X70 material, relative to A36 and X52, is a result of the higher microaffecting content (Ti and Nb), which aids in grain refinement during thermo-mechanical controlled processing. Tensile tests of the three steels were performed in air according to ASTM E8/EBM Standard Test Methods for Tension Testing of Metallic Materials [44] by use of a 250 kN servohydraulic test frame. The average mechanical properties from three tests, and the corresponding standard deviations of the measured properties are shown in Table 2.

2.2. Fatigue crack propagation measurements

Baseline tests performed in air and simulated fuel-grade ethanol with the test procedure summarized here are reported in greater detail elsewhere [45]. Compact tension (CT) specimens (Fig. 2) were machined from the three steels (in the LT orientation) in accordance with ASTM E647 Standard Test Method for Measurement of Fatigue Crack Growth Rates [46]. The pipeline steel specimens were machined from curved pipe sections that were not flattened prior to specimen sectioning. The X70 pipe wall section (6.60 mm) limited the “B” dimension (specimen thickness) to 5.715 mm due to pipe curvature. All specimens were made to this thickness so that the stress state of the crack would be consistent among the tests. Fatigue precracking and crack propagation measurements were performed with the compliance technique. Crack mouth opening displacements (CMOD) were used for compliance measurements. Displacement was measured in the load line with a coated 6.35 mm gauge length crack-opening displacement gauge attached to integral knife edges machined at the crack mouth. An RTV coating was applied to prevent degradation of the clip gauge in the corrosive test environments. Calibration of the gauge was performed after application of the coating and verified between

<table>
<thead>
<tr>
<th>Alloy</th>
<th>C</th>
<th>Si</th>
<th>Cr</th>
<th>Ni</th>
<th>Mn</th>
<th>Cu</th>
<th>Mo</th>
<th>Nb</th>
<th>Ti</th>
<th>Al</th>
<th>V</th>
<th>S</th>
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</thead>
<tbody>
<tr>
<td>A36</td>
<td>0.22</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
<td>0.09</td>
<td>0.23</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>X52</td>
<td>0.070</td>
<td>0.195</td>
<td>0.030</td>
<td>0.020</td>
<td>1.505</td>
<td>0.050</td>
<td>0.004</td>
<td>0.021</td>
<td>0.001</td>
<td>0.029</td>
<td>0.003</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>X70</td>
<td>0.050</td>
<td>0.185</td>
<td>0.043</td>
<td>0.017</td>
<td>1.505</td>
<td>0.030</td>
<td>0.01</td>
<td>0.084</td>
<td>0.015</td>
<td>0.032</td>
<td>0.01</td>
<td>0.006</td>
<td>0.012</td>
</tr>
</tbody>
</table>
tests. Precracking was performed in air at 20 Hz, and loads were incrementally shed so that initial loads during crack propagation studies were greater than final loads during fatigue precracking. Tests were performed in a 100 kN closed loop servohydraulic test frame with a sinusoidal waveform and constant force \( P \) ratio \( R = P_{\text{min}}/P_{\text{max}} = 0.1 \). Environmental testing was performed with a loading frequency \( f \) of 0.1 Hz. Liquid pipelines likely experience higher \( R \) and lower loading frequency than those reported here; however, we selected a particular mechanical condition to evaluate the effect of bacteria under accelerated conditions. Stress-intensity amplitude was controlled by software and continually increased during testing at a rate, \( C = 0.15 \text{ mm} / \text{C}0^{1/2} \), which is the normalized \( K \)-gradient.

2.3. Biological culturing and fatigue testing

Microbiological species including *Acetobacter* sp. and a sulfate-reducing consortium that included *Desulfovibrio vulgaris* and *Clostridia* sp. were cultivated from industrial ethanol containment-tank samples [47]. These tanks contained fuel-grade ethanol and water. *Acetobacter* sp. were maintained in a medium containing yeast extract (0.5 g L\(^{-1}\)), peptone (0.3 g L\(^{-1}\)), and sodium chloride (1 g L\(^{-1}\)) in distilled water (adapted from Lisdiyanti et al. [48]). Ethanol (5% by volume) was added as a carbon source. *Acetobacter* sp. are aerobic bacteria that metabolically oxidize ethanol into acetic acid. The sulfate-reducing consortium was maintained in a modified Postgate B medium that included potassium dihydrogen phosphate (0.5 g L\(^{-1}\)), ammonium chloride (1 g L\(^{-1}\)), calcium sulfate (1 g L\(^{-1}\)), magnesium sulfate 7-hydrate (2 g L\(^{-1}\)), yeast extract (1 g L\(^{-1}\)), ascorbic acid (0.1 g L\(^{-1}\)), thioglycollic acid (0.1 g L\(^{-1}\)), and iron sulfate 7-hydrate (0.5 g L\(^{-1}\)) [49]. Ethanol (2% by volume) was added as a carbon source after 0.2 \( \mu \text{m} \) filtering. The SRB consortium was maintained under a nitrogen headspace. This consortium included *Desulfovibrio vulgaris* and *Clostridia* sp. *Desulfovibrio vulgaris* sp. are anaerobic spore-forming bacteria capable of reducing sulfate to sulfide in an eight electron energy transfer, and these microbes have been associated with fuel-contaminated environments as well as iron transformations [42,50]. *Clostridia* sp. are anaerobic, spore-forming bacteria capable of various metabolisms [51]. It is expected that APB and SRB could be found together as a consortium in a biofilm on a steel surface since aerobic species (APB) could thrive in an oxic environment and consume oxygen, thereby providing an anoxic environment suitable for anaerobic SRB.

In this study, the impact of APB and SRB metabolisms on crack growth were examined. Cultures were inoculated into test solutions in a controlled laboratory environment. Test solutions were
poured into a 6 L test vessel, and a mechanical reaction frame containing the C(T) specimen was lowered into the test environments. Care was taken when preparing the testing environment, because past work has demonstrated that metabolic transients can affect the applicability of fatigue test results based on the concept of similitude [31]. To avoid such transients in this study, microbial cultures were maintained in the stationary phase, i.e., the growth rate and death rate of bacterial cultures are equal. Cells were counted with a Petroff-Hausser cell counting chamber and a phase-contrast microscope before and after fatigue-crack propagation testing. Cell densities were maintained on the order of $10^7$ cells per mL of media for inoculated tests. Solution acidity was measured with a pH probe at the beginning and end of fatigue testing.

Relevant conditions of the test solution, including pH and cell counts, are reported in Table 3.

Fig. 3 shows a schematic of the mechanical reaction frame that was implemented to completely submerge C(T) specimens into test environments. The reaction frame, which was made of high-strength stainless steel, was electrically isolated from the C(T) specimen with Teflon washers and ceramic loading pins to prevent galvanic interactions. The test chamber and seals were made of polymeric materials with good chemical resistance to ethanol. Air-tight seals were maintained throughout testing to maintain relatively constant test conditions, namely to prevent solution evaporation or contamination. Precracked C(T) specimens were submerged in the test environment under free-corrosion conditions. The precrack was grown for a period of ~24 h before commencing data collection. APB test solutions were exposed to air in the headspace above the test solutions, but were not mechanically aerated. SRB test solutions were covered with approximately 25 mm of vegetable oil and purged with high purity N₂ gas to maintain anaerobic conditions. Anaerobic conditions were confirmed in the test chamber by the redox indicator resazurin. This compound is an oxygen-reduction indicator that turns solutions to a reddish hue if proper deaeration is not maintained. Fatigue testing in the APB and SRB environments last ~5 days per experiment.

2.4. Characterization procedures

C(T) specimens were sonicated in 200 proof ethanol immediately following fatigue propagation testing. Fracture surfaces were liberated in liquid nitrogen after cleaning and then stored in a desiccated container. A scanning electron microscope (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) was used to evaluate fracture surfaces and remnants of biological activity (e.g., mineral deposits). An accelerating voltage of 15 kV and a light-element detector window were used for chemical analyses. EDS spectra were not quantitative due to the unavailability of appropriate spectrographic material standards used for chemical analysis of corrosion product and biological materials. Fracture specimens were not coated (e.g., with carbon or gold sputter coat) prior to examination.

3. Results and discussion

This study builds on a previous study that reported effects of simulated fuel-grade ethanol (SFGE) on the fatigue crack propagation in A36, X52, and X70 tank and pipeline steels [45]. That study showed that each of these steels may experience degradation due to superimposed mechanical fatigue and ethanol stress-corrosion cracking by fatigue loading during transport of FGE. Jain [41] found through electrochemical studies that MIC produced accelerated corrosion rates and localized corrosion on A36, X52, and X70 steels in FGE environments using the same culture strains and material heats studied here. That study also suggested that MIC may occur simultaneously to fatigue and stress-corrosion cracking and these findings formed the basis of the research questions addressed herein.

Due to the hygroscopic nature of ethanol, water-rich phases that could harbor microbiological life are generally not expected. However, upset conditions such as those experienced during hydrostatic testing are conducive to microbiological growth [27]. In addition, the presence of any significant or remnant water layer (i.e., the Helmholtz Layer) on a steel surface can provide an essential need for microbial/biofilm life. The introduction of water (artificially and/or otherwise) into a pipeline or tank that contain

### Table 3

<table>
<thead>
<tr>
<th>Test number</th>
<th>Initial cell count (#/mL)</th>
<th>Final cell count (#/mL)</th>
<th>Initial solution acidity (pH)</th>
<th>Final solution acidity (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X70-5 (Control Test Solution)</td>
<td>0</td>
<td>$9 \times 10^6$</td>
<td>6.60</td>
<td>4.82</td>
</tr>
<tr>
<td>X70-6 (APB + Test Solution)</td>
<td>$2 \times 10^7$</td>
<td>$3 \times 10^7$</td>
<td>3.60</td>
<td>3.58</td>
</tr>
<tr>
<td>X70-7 (SRB + Test Solution)</td>
<td>$8 \times 10^7$</td>
<td>$6 \times 10^7$</td>
<td>6.5–7.0</td>
<td>6.5–7.0</td>
</tr>
<tr>
<td>X52-6 (Control Test Solution + 1 g L⁻¹ glutaraldehyde)</td>
<td>0</td>
<td>~</td>
<td>6.0–6.5</td>
<td>6.0–6.5</td>
</tr>
<tr>
<td>X52-5 (APB + Test Solution)</td>
<td>$2 \times 10^7$</td>
<td>$3 \times 10^7$</td>
<td>3.35</td>
<td>3.36</td>
</tr>
<tr>
<td>X52-7 (SRB + Test Solution)</td>
<td>$7 \times 10^7$</td>
<td>$6 \times 10^7$</td>
<td>6.5–7.0</td>
<td>6.5–7.0</td>
</tr>
<tr>
<td>A36-5 (APB + Test Solution)</td>
<td>$6 \times 10^7$</td>
<td>$5 \times 10^7$</td>
<td>3.15</td>
<td>3.12</td>
</tr>
<tr>
<td>A36-4 (SRB + Test Solution)</td>
<td>$4 \times 10^7$</td>
<td>$5 \times 10^7$</td>
<td>6.5–7.0</td>
<td>6.5–7.0</td>
</tr>
</tbody>
</table>

APB Test Solution: 5% ethanol (vol), 1 g L⁻¹ NaCl, 0.5 g L⁻¹ yeast extract, 0.3 g L⁻¹ peptone, balance H₂O.
SRB Test Solution: 2% ethanol (vol), balance Postgate B medium, Purged with N₂.

* Values are approximate and were measured with pH paper. Other values were measured with pH probe.
remnants of FGE establishes an ideal situation for MIC since water and a carbon source (ethanol) are necessary. Electron donors and acceptors are also needed for metabolic processes and can be provided by trace contaminants and H₂ found in the host steel and in the fuel and water in the pipelines. Therefore, if biofilms are established during the hydrostatic testing, there is a possibility that they remain intact after fuel transport recommences. The biofilms may harbor localized environments significantly different from those in the pipeline and result in surface pitting [7], for example. Given that pits are likely sites for fatigue crack initiation [39], and that microbiological species living within biofilms can produce chemical species that degrade the mechanical integrity of steel, the following results are applicable for describing upset conditions in the presence of APB (e.g., Acetobacter sp.) and SRB (e.g., Desulfosporo-rinus sp.) in ethanol environments where water contents are initially high enough to promote and sustain microbiological life. However, the chemical species produced by these bacteria are not specific to ethanol, virtually any hydrocarbon makes for an ideal carbon source for a diversity of microbiota, and thus these results may have broader applicability to other biofuels and the oil and gas industry.

3.1. Crack growth in APB environment

A drastic increase in fatigue-crack propagation rates is induced by APB (shown in Fig. 4) for all three of the steel alloys tested here when compared to air and simulated FGE environments [45]. Note that at low levels of ΔK (the stress-intensity-factor amplitude) there is a strong dependence of crack growth rate on ΔK. At a ΔK level of 20 MPa m⁻¹/² there is an approximate 25-fold increase in the rate of crack propagation, relative to air, in the two pipeline steels. Crack growth data of all three materials tested in the APB solution essentially overlay one another below ΔK values of approximately 25 MPa m⁻¹/². A significant transition in the slope of da/dN vs. ΔK occurs near this level, indicating a wide variation in Paris Law exponents and ΔK-independent crack growth rates. The presence of this plateau region is explained as being related to mass transport limitations of chemical species at the crack tip [38]. The plateau limits crack growth at intermediate levels of ΔK to approximately 2 × 10⁻⁶ m/cycle in the A36, and 1.5 × 10⁻⁶ m/cycle in the two pipeline steels. Note that the pH was lowest in the A36 APB test (due to APB-produced organic acids), which may contribute to the higher observed crack growth rates in the plateau region, although the increase in crack growth, relative to air, is still lowest in A36. As ΔK values increase beyond the plateau, the da/dN values resume the increasing trend in each material, where they converge towards the crack growth rate data measured in air. This behavior can be explained as the purely mechanical damage component begins to dominate at the higher ΔK levels.

Acidity values and results of bacterial cell counts of the APB test solutions are included in Table 3, which represent test solution conditions at the start and end of each fatigue test. Acetobacter sp. oxidized ethanol into acetic acid and lowered the pH of the test solutions from an initial "control" solution pH of approximately 6.6 to pH levels of 3.1 to 3.6. Note that pH changed very little during the course of the tests in APB solutions and that bacterial cell counts remained on the order of 10² cells per mL. Thus, significant transients in crack growth behavior were not expected due to the relative constancy of the cell counts, and their subsequent metabolic reactions.

Crack-growth rates were measured in X70 in an ethanol test solution “control” that was not inoculated with microbiological species as shown in Fig. 4(c). Differentiation between the effect of the high water content in the ethanol solutions, which promotes rapid microbiological growth, and the effect of the microbes themselves is the basis for a control test. The control solution contained ethanol, water, chloride, yeast extract, and peptone, just as the test solution (essentially a minimum microbial growth medium). A previous report indicated that increases in the water content of ethanol increases fatigue crack growth rates in steel [52]. While it is known that aqueous chloride solutions can influence crack growth, an effect of yeast extract and peptone is not expected. However, contamination of the X70 control (test X70-5 in Table 3) became evident as microbes, including APB, are ubiquitous throughout soils and any number of environments in nature. This natural abundance made it difficult to run a true control solution. Cell counting in this control test showed a significant concentration of putative APB cells in the solution (~10⁶ cells per mL) albeit at concentration levels significantly lower than that of the inco-

![Fig. 4. Fatigue crack propagation rates of (a) A36, (b) X52, and (c) X70 in various test environments including air, simulated fuel-grade ethanol (SFGE), acid-producing bacteria (APB), and sulfate-reducing bacteria (SRB). Air and SFGE data are adapted from Ref. [45].](image-url)
lated solutions maintained in the stationary phase. This concentration was enough to lower pH from 6.60 (control condition) to 4.82 after contamination. Still, the crack growth rates in this solution are significantly lower than in the inoculated APB solution. This suggests that decreases in pH shift the plateau up and to the left, i.e., to promote the apparent effect of increasing crack growth rates at significantly lower ΔK values.

A powerful protein cross-linker, glutaraldehyde is often used as an effective biocide in industrial operations in the oil and gas industry. It was included in a control test of X52, at a concentration of 1 g/L, to determine its effectiveness in preventing growth of the APB; though, no APB were added to the test solution. Crack growth rates in this solution are shown in Fig. 4(b). The experiment that contained glutaraldehyde additions (test X52-6 in Table 3) showed the lowest crack growth rates of all tests in the biological test media. Acidity level did not change significantly during the control test containing the biocide glutaraldehyde even though microbes such as APB are ubiquitous and readily contaminated the X70-5 control test. Also, test solutions did not become cloudy as in other test solutions. The effect of this biocide appears promising in control of the APB species, but effects would have to be weighed upon the pipeline and tank-stored fluids as well. It is also unclear how glutaraldehyde would affect other microbes. Note also that the plateau effect had significantly lower crack growth rates in this test. This test likely represents the combined effect of corrosion fatigue and possible environmental embrittlement of steel by the aqueous ethanol solutions used to grow the bacteria. Increases in environmental crack growth beyond this must have been due to bacteria metabolic products.

3.2. Crack growth in SRB environment

In general, the highest increase in fatigue-crack propagation rates in the pipeline steels was induced by the SRB, as shown in Fig. 4. Crack growth was accelerated by over 40-fold at the lower stress intensities. However, the effect of SRB was not as potent in increasing crack growth rates in the A36 steel, which may have lower hydrogen damage susceptibility due to its inherently lower strength compared to that of the two pipeline steels (Table 2). The SRB produced higher crack growth rates at lower stress intensity values than the APB environments in all three materials, which indicates a significantly lower threshold ΔK level for the active damage mechanism. The crack growth data of X52 and X70 overlay one another below 20 MPa m^{1/2}. Note that in the A36 steel however, the most distinct environmental cracking threshold is apparent where the SRB sharply increased crack growth at a ΔK value of approximately 15 MPa m^{1/2}. Such a sharp increase in crack growth is not observed in any of the other tests. This distinct threshold on a plot of dα/dN versus ΔK is normally associated with activation of either an SCC damage mechanism [53] or hydrogen-assisted damage mechanism [54]. In the present case, it would likely be a

![Fig. 5. Secondary electron SEM images of X52 fracture surface after testing in acid producing bacteria and sulfate reducing bacteria solutions showing intergranular fracture at low ΔK and transgranular fracture at intermediate ΔK. Chemical analysis locations are indicated, including corresponding EDS spectra showing enrichment of organic elements in corrosion products.](image-url)
hydrogen-damage mechanism related to biotic H$_2$S production, as discussed below.

The increase of crack growth rate in SRB environments examined here exhibit the plateau behavior described by Thomas et al. [38] at intermediate stress intensities much like the APB environments. Crack growth rates peak in the region between $\Delta K$ levels of approximately 30 MPa m$^{1/2}$ to 35 MPa m$^{1/2}$. Thomas et al. demonstrated that the plateau behavior was related to the H$_2$S content in high-strength microalloyed steels [38]. This plateau was shown to shift to higher crack growth rates and lower stress intensity levels as the H$_2$S content increased, and eventually reached a behavior where a steep threshold sharply transitioned into a linear variation of $da/dN$ with $\Delta K$ as H$_2$S became saturated. Based on that model, H$_2$S saturation was not realized in the SRB environments here, which probably have concentrations well below 600 ppm (level established in [38]), although measurements would be required to substantiate the actual concentrations. There is some consensus that 50–200 ppm H$_2$S is representative of SRB at the surface of metal [55], which would be well below the apparent values for saturation.

3.3. Fracture behavior

The fracture behavior was evaluated by examining crack surfaces with SEM. In a benign air environment these materials exhibit the typical flat transgranular fracture where indications of fatigue striations associated with each $da/dN$ cycle are clearly evident. Fig. 5 shows the fracture appearance of X52 tested in the APB and SRB environments. The images are oriented so that the crack growth direction is from right to left and the crack front from top to bottom. The plane of the crack is perpendicular to the image. Fracture-surface locations corresponding to low and intermediate levels of $\Delta K$ were selected for analysis due to the differences in crack-growth rate behavior observed in Fig. 4. Low $\Delta K$ values correspond to the behavior of rapid crack-growth rate increases with increasing $\Delta K$, i.e., less than approximately 20 MPa m$^{1/2}$. Intermediate $\Delta K$ values correspond to the plateau region.

In comparison to the fracture appearance in air, at the low levels of $\Delta K$, the embrittling effect is quite clear in both APB and SRB environments. The intergranular nature of the environmental fracture is apparent. Qualitative estimates of the fracture in these low $\Delta K$ regions suggest that approximately half of the fracture surface area is intergranular and half transgranular in an APB environment. Fractures appear to have more of an intergranular component in the SRB environment as indicated qualitatively by a greater overall surface ratio. Note that the intergranular facets have a corroded appearance after exposure to the APB solution whereas they are relatively undamaged after exposure in the SRB solution. This suggests that the anodic dissolution occurred along the fracture in the APB solution, although it is not apparent at what point this occurred during the test, since the low $\Delta K$ portion of the crack was exposed to test solution for several days after the crack propagated through this region. Chemical analyses in the intergranular regions suggest that Cl and S are enriched in the corrosion product on the fractures. Fractures have an entirely different appearance at the intermediate $\Delta K$ levels within the plateau region. The fracture appearance is exclusively transgranular. The features are again mottled on surfaces exposed to the APB environment, whereas they are clearly serrated in the SRB environment. The fracture surfaces of $\Delta K$ levels above the plateau are also transgranular in both environments. In the SRB environment, the fatigue striations grew with the level of $\Delta K$, as expected by the increase in $da/dN$.

3.4. Biofilms associated with APB and SRB environments

Upon removal of the (C(T)) specimens from the testing environments, biofilms were readily apparent on the specimen surfaces and were quite extensive. Corrosion product was also apparent and was associated with the biofilms. If the biofilms were not removed before drying out, they became firmly adhered to the specimen surfaces quite rapidly. Fig. 6 shows the typical appearance of two specimens immediately following removal from the test solutions. The APB biofilm had a light-colored opaque appearance, and the steel beneath it had begun to oxidize rapidly upon removal, which is evident by the apparent rusty color under the biofilm. The SRB biofilm was black in color and appeared to be deposited in a layer much thicker than the APB film. This biofilm became quite tenacious after drying, even to the extent that sonication in pure ethanol solution would not remove it from the specimen surface. The top half of the SRB test specimen shown in Fig. 6 was wiped with ethanol immediately after removal and shows extensive oxidation under the location of the biofilm. The tenacity of the SRB biofilm in pure ethanol is of concern. If such a biofilm were to form under upset conditions where water may be present, the flow of ethanol fuel following the upset may not necessarily dissolve the biofilm, based on the experience with sonication in pure ethanol. Also given that the SRB species here are spore formers, they may go dormant until conditions are more conducive for them to begin growing again.

It is noteworthy that on the crack plane near the external surface of the (C(T)) specimen, biological debris resulting from the APB biofilm was apparent, as shown in the SEM micrograph in Fig. 7. Similar debris was apparent all along the fracture surface, as indicated in EDS analysis locations in Fig. 5. Surviability of microbiological species is unclear in a crack that fully unloads and closes, and should be a topic for prospective investigations on the relationship between MIC and growing tight fatigue cracks. Though given the micron size nature of most microbes, combined with possible formed pits in the steel, survivability is likely not an...
issue across the unload and closure regime. Mass-transport conditions at a submerged fatigue-crack mouth have been described in numerous studies, but the effect of flow on the ingress and egress of bacteria from the crack is not apparent.

This study did not consider the important aspect of corrosion fatigue-crack initiation. The likely fatigue-crack initiation sites can be proposed based on evaluation of the biofilms on the external surfaces of the C(T) specimens, i.e., not the crack plane. Figs. 8 and 9 characterize the regions in the immediate vicinity of the crack on the external surface of the X52 C(T) specimens tested in APB and SRB solutions, respectively. Pitting susceptibility of steel in simulated FGE decreases if water content becomes higher than about 10% [56]. Based on that finding, and the fact that APB solutions in this study had high water and low ethanol concentrations, the pitting behavior observed on C(T) specimens (shown in Fig. 8) can likely be attributed to the presence of acetic acid secreted by the APB. Acetic acid produced by bacteria has been shown to attack the matrix of pipeline steel at inclusion sites, resulting in a deep-pitting corrosion [30]. The secondary electron image in Fig. 8 reveals significant corrosion pitting in the central location of the image. The chemical analyses qualitatively show that the regions surrounding the pits have elevated carbon, oxygen, and chlorine content, whereas analysis at the center of the pits reveals mainly iron. The apparent organic debris also appears enriched in those elements. The presence of differential aeration cells likely resulted in some of the pitting, since the biofilms were not necessarily continuous on the surface. Deep pitting appeared to be related to some of the APB biofilms. The apparently organic residue associated with the SRB biofilm shown in Fig. 9 is enriched in carbon, oxygen, and chlorine and devoid of iron. Some sulfur enrichment is also notable, as would be expected with sulfate reducing species. The composition maps also show that Mn enrichment may coincide with the sulfur, indicating the presence of MnS inclusions. A pit was apparent at one location of elevated Mn and S. The organic residue along the bottom of the image tends to align itself along the surface scratches. Areas of increased surface roughness, which are quite common, would be expected to be ideal attachment points for the biofilms inside a pipe or tank, as reviewed elsewhere [7]. Jain has developed electrochemical-based models that describe pit formation on these materials in the tested culture media [41]. That study was performed on bulk specimens and the electrochemical measurements would have less relevance to the sharp fatigue crack tip tested here due to polarization, migration, and advection–diffusion behavior.

The possibility of both APB and SRB coexisting in a biofilm should be considered. Both types of microbes were present in the tanks from which our samples were acquired [47]. Also, other work has shown that an increase in metabolic products that lower pH, such as acetic acid, also increase the levels of H₂S and HS⁻ by increasing Fe²⁺ availability for SRB [57]. Based on that finding, we suggest that mixed cultures of APB and SRB in ethanol fuels such as those tested individually here, may in fact promote an even worse case for inducing fatigue damage. Culturing a mixture of species was difficult in the present study and requires further development before fatigue testing can be conducted.

3.5. Hydrogen damage mechanisms

The APB and SRB environments tested here resulted in a typical crack growth behavior that is characterized by: (1) a steep increase in crack growth rate at low ΔK followed by (2) a plateau region of ΔK-independent crack growth rate across the intermediate values of ΔK. The behavior has been described for low-alloy steels subjected to fatigue loading in biotically produced H₂S environments [38] and an X70 pipeline steel subjected to fatigue loading with impressed cathodic protection [58]. Fig. 10 shows the latter case, because an X70 grade steel was also evaluated in the current work. The data set at −1.03 V̸C̸E provides an environment where the test specimen is cathodically charged with abiotic hydrogen. The APB data here coincide very well with the abiotic source of hydrogen, as do the SRB data, although the crack growth rates are higher in the latter case. Both the APB and SRB provide a source of hydrogen, which causes embrittlement of steel through hydrogen adsorption (Hₐds) followed by hydrogen entering into solution (Hₐsol), where it will diffuse to the plastic zone ahead of the crack where stress triaxiality is high. The slowest (rate-limiting) step in this process controls the rate of crack propagation. The adsorption and absorption of hydrogen into the iron lattice are governed by Reactions (1) and (2):

\[
\begin{align*}
H_{\text{ads}} & \rightarrow H_{\text{ads}} + H_{\text{ads}} \\
H_{\text{ads}} & \rightarrow H_{\text{ads}}
\end{align*}
\]

The APB inoculated in the ethanol solutions in this work produce acetic acid by nature. Acetic acid is a weak acid, and a potential source of hydrogen to embrittle the crack tip upon adsorption on the crack tip, dissociation, and finally absorption into the iron lattice, where it can migrate to the plastic zone. The cathodic reactions on pipeline steel in the presence of acetic acid have been reviewed in Ref. [59], and are described by Reactions (3) and (4):

\[
2H^+ + 2e^- \rightarrow H_2
\]
The embrittling effect of the dissociation of molecular hydrogen into adsorbed atomic hydrogen on steel is well known and is apparently readily available based on Reactions (3) and (4). However, acetic acid in the presence of hydrated ethanol can have an inhibiting effect related to the surface adsorption of the acetate ion [60]. The adsorption of acetate along the advancing fatigue crack may play a role in the hydrogen uptake process and may also decrease contribution of the anodic dissolution to the overall rate of crack advance. Additions of sodium can accelerate the adsorption on steel and increase the hydrogen evolution reaction [61]. The test solutions used here contained 1 g L\(^{-1}\) NaCl, which may have participated in advancing the hydrogen evolution to a greater extent than merely cathodic charging (abiotic \(H_2\) charging at \(-1.03\) \(V_{SCE}\) in Fig. 10). This is supported by the observation of extensive chloride deposition on the fracture surfaces reported in Fig. 5. The kinetics of the acetate adsorption on newly exposed metal during fatigue loading would govern the contribution of dissolution to the true corrosion fatigue. A pH-dependent behavior in APB environments is also shown in Fig. 10. Note that the lower pH condition still does not compare favorably with a case of true corrosion fatigue (X70 in 3.5% NaCl at \(-0.7\) \(V_{SCE}\) potential), and the apparent threshold (sharp increase in crack growth rate) and plateau effect are still noted. The line of true corrosion fatigue runs approximately parallel to air data, indicating that dissolution is a relatively constant contribution to enhanced crack growth irrespective of \(\Delta K\). Therefore, it appears that decreasing pH increases available hydrogen for adsorption, and that crack tip embrittlement is the mechanism, rather than increasing the crack advance by an anodic dissolution mechanism.

Based on the relative similarity in the observed plateau behavior in both the APB and SRB solutions, the effect of the SRB on anodic dissolution and its resulting contribution to the crack growth is minimal. The obvious explanation for the increased crack growth in SRB environments relative to APB environments is the presence of sulfur in the cathodic reaction. The SRB, when present in neutral anaerobic environments, induces the cathodic reaction according to Reaction (5) [7] or more appropriately for the adsorption into steel by Reaction (6) [55]:

\[
\begin{align*}
H_2S + e^- & \rightarrow HS^- + \frac{1}{2}H_2 & \text{(5)} \\
H_2S + 2e^- & \rightarrow HS^- + H_{ads} & \text{(6)}
\end{align*}
\]

\(H_2S\) has greater thermodynamic stability than \(HS^-\) at pH values below 7 [55] which corresponds to the SRB solutions here (Table 3).
The corrosion reaction becomes poisoned by the presence of sulfide, i.e., the rate of hydrogen recombination according to Reaction (3) is reduced. The presence of the hydrogen sulfide on the surfaces is indicated by the overall higher sulfur intensity on the surfaces near the biofilms tested in the SRB solution (Fig. 8) relative to the surfaces of those specimens tested in APB solution (Fig. 9).

4. Conclusions

1. Microbiological species, including a sulfate-reducing bacterial consortium (Desulfosporosinus sp. and Clostridia sp.) and Acetobacter sp., were cultivated from an FGE environment and inoculated into test media where fatigue testing shows marked increases in the fatigue crack propagation rates in the storage tank and pipeline steels evaluated here.

2. The acid producing bacteria produced acetic acid, which dissociates, and provides a source of hydrogen for fatigue-crack tip embrittlement. Increases in solution acidity increase the rate of crack growth and decreases the apparent threshold stress intensity for environmental cracking. The hydrogen can also serve as a strong electron donor for microbiota present to further propagate both metabolism and MIC.

3. Glutaraldehyde may inhibit growth of the Acetobacter sp. and other microbial species. The compound prevented APB contamination of the control test; thus, eliminating their embrittling effect during testing of X52 pipeline steel. Further testing is required to determine the efficacy of this compound.

4. Sulfate reducing bacteria evaluated here produced the highest observed crack growth rates as a result of sulfide-enhanced hydrogen embrittlement.

5. The environmentally assisted fracture morphologies associated with the acid producing bacteria and sulfate reducing bacteria vary, depending on stress-intensity amplitude. Low values of stress-intensity amplitude result in predominantly intergranular fracture, whereas intermediate and high values of stress-intensity amplitude result in a transgranular fracture mode.

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