Symbiosis of Sulfate-Reducing Bacteria and Total General Bacteria Affects Microbiologically Influenced Corrosion of Carbon Steel

Juxing Jin 1, Yingchao Li 1,*, Huaiwei Huang 2, Yong Xiang 2 and Wei Yan 3

1 Beijing Key Laboratory of Failure, Corrosion and Protection of Oil/Gas Facility Materials, College of New Energy and Materials, China University of Petroleum-Beijing, 18 Fuxue Road, Changping, Beijing 102249, China
2 College of Mechanical and Transportation Engineering, China University of Petroleum-Beijing, 18 Fuxue Road, Changping, Beijing 102249, China
3 Unconventional Petroleum Research Institute, China University of Petroleum-Beijing, 18 Fuxue Road, Changping, Beijing 102249, China; yanwei@cup.edu.cn
* Correspondence: liyc@cup.edu.cn

Abstract: The effects of the symbiosis of sulfate-reducing bacteria (SRB) and total general bacteria (TGB) on the microbiologically influenced corrosion (MIC) of carbon steel were investigated in this research. The SRB was the main corrosive bacterium, and TGB induced slightly general MIC. The symbiosis of SRB and TGB induced more severe MIC and pitting corrosion than SRB. The main corrosion products were FeS, Fe₂O₃, and FeOOH. The presence of TGB facilitates MIC and pitting corrosion by providing a locally anaerobic shelter for SRB. An MIC mechanism of the symbiosis of SRB and TGB was proposed.

Keywords: symbiosis; SRB; TGB; MIC pitting; EET

1. Introduction

Microbiologically influenced corrosion (MIC) has been studied for over 100 years due to its practical significance and complex mechanisms [1]. It is a primary cause of various corrosion failures, including pitting, perforation, and cracking [2–5]. Microorganisms tend to adhere to solid surfaces, such as metals [6]. Biofilms, formed at the interface between the bulk solution and the metal, alter physical and chemical properties including local pH levels, oxygen concentration, and ion species and concentrations. These changes significantly impact the corrosion behavior of the metal substrate [7–10].

Sulfate-reducing bacteria (SRB) are widely considered the most representative microorganisms in the anaerobic environments typical of the oil and gas industry and are extensively studied in MIC research [11–14]. The abundant sulfate naturally present in these environments serves as electron acceptors for SRB, facilitating their growth and metabolism. This interaction significantly accelerates MIC in iron materials, such as carbon steel and stainless steel. The mechanism of MIC induced by SRB has been thoroughly investigated [15–19]. Gu et al. introduced the biocatalytic cathodic sulfate reduction (BCSR) theory, which elucidates the role of SRB in the MIC process from bioenergetic and bioelectrochemical perspectives [20]. Subsequent studies have expanded on the BCSR theory, particularly regarding the electron transfer process [21,22]. Since insoluble iron can act as an electron donor, SRB utilize electrons released from iron dissolution for sulfate reduction in their cytoplasm, employing biocatalysts. This process requires electron transfer across the cell wall, from the iron to the cytoplasm, a mechanism known as extracellular electron transfer (EET) [19,23]. EET can be facilitated by two methods: direct electron transfer (DET), which includes direct contact and conductive pili attaching to the iron surface to harvest electrons [22], and mediated electron transfer (MET), which relies on soluble redox...
mediators secreted by microorganisms [24,25]. The BCSR theory has shed light on how SRB accelerate the corrosion of iron. According to this theory, MIC can be categorized into EET-MIC and metabolite MIC (M-MIC) [19,26].

In the oil and gas field, various microorganisms coexist, forming a multispecies microbial community [27,28]. Numerous microbes have been detected in this industry [29,30]. Notably, anaerobic bacteria are found within aerobic environments because the outer aerobic biofilm acts as an oxygen diffusion barrier, creating local anaerobic conditions underneath. Additionally, the exchange of metabolites between different species facilitates energy transfer and improves the living conditions within the microbial community [31], promoting coexistence. This symbiosis complicates the interpretation of MIC mechanisms. For instance, the coexistence of iron-oxidizing bacteria (IOB) and sulfate-reducing bacteria (SRB) has been shown to facilitate pitting corrosion and alter the structure and metabolic activities of biofilms [32]. Liu et al. observed that in oxygen-containing environments, IOB enhanced the growth of sessile SRB but suppressed the growth of planktonic SRB when cultured together, thereby enhancing pitting corrosion [33]. Additionally, Unsal et al. demonstrated that acid-producing bacteria (APB), SRB, and general heterotrophic bacteria (GHB) formed rough biofilms on coupons, resulting in pitting corrosion [34]. Interestingly, the coexistence of Pseudomonas aeruginosa (a denitrifying bacterium) and Desulfovibrio vulgaris (SRB) reduced the MIC of cast iron compared to when SRB were present alone [35]. Thus, the mechanisms by which bacterial symbiosis affects MIC in metals remain enigmatic.

In oil and gas systems, a consistent co-detection of anaerobic bacteria, specifically sulfate-reducing bacteria (SRB) and aerobic bacteria, generally referred to as total general bacteria (TGB), has been observed. Although TGB are primarily aerobic and have not been extensively studied for their role in microbiologically influenced corrosion (MIC), they are frequently found in consortia with other species. This paper investigates the effects of the symbiosis between SRB and TGB on the MIC behavior of carbon steel. We studied the MIC mechanisms of carbon steel when exposed to TGB and SRB, both separately and in combination. Weight loss measurements were employed to analyze the general corrosion rate. The corrosion morphology of the carbon steel samples was examined using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). The chemical compositions of the corrosion products were analyzed with energy-dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS). Electrochemical techniques were utilized to elucidate the corrosion processes. We propose a mechanism by which symbiosis influences the MIC of carbon steel, enhancing our understanding of these complex interactions.

2. Experimental Section

2.1. Materials and Pretreatment

In this study, ordinary 20# carbon steel coupons (dimensions: 50 × 10 × 3 mm and 10 × 10 × 4 mm) were employed for weight loss measurements and electrochemical tests. The chemical composition (wt%) of the 20# steel included C (0.17–0.24), Si (0.17–0.37), Mn (0.35–0.65), Cr (≤0.25), Ni (≤0.25), Cu (≤0.25), P (≤0.035), and S (≤0.035), with the balance being Fe. All coupons were progressively abraded with 400-, 800-, and 1200-grit silicon carbide papers, rinsed with acetone and anhydrous ethanol, and dried with high-purity nitrogen (N2). Each rinsing and drying process lasted for 10 s. Coupons were stored in a vacuum chamber at 25 °C for no more than 4 h before being used in corrosion tests [19].

2.2. Cultivation and Inoculation

SRB and TGB were isolated from a produced water sample collected from a PetroChina oil field and selected for multispecies biofilm formation. The SRB seed culture was grown in ATCC 1249 Modified Baar’s medium with the following composition (g/L): CaSO4 (1.0), K2HPO4 (0.5), MgSO4·7H2O (2.0), NH4Cl (1.0), sodium citrate (5.0), sodium lactate (3.5), and yeast extract (1.0). The TGB seed culture was cultivated in a medium containing the following (g/L): beef extract (1.0), peptone (5.0), glucose (1.0), and yeast extract (1.0). Both
media were sterilized by autoclaving at 121 °C for 20 min. The pH of both media was
adjusted to 7.0 by 1M NaOH. The SRB medium was subsequently purged with high-purity
N₂ (99.999% vol.) to eliminate oxygen. For symbiosis tests, equal volumes of SRB and
TGB seed cultures were introduced into vials containing their respective media and were
incubated at 38 °C in anaerobic vials. Each vial contained three replicate coupons, with
each experiment being duplicated for reproducibility. All experiments were conducted at
38 °C over a period of eight days inside an anaerobic glove box that was sparged with N₂
for 45 min. Prior to incubation, the UV light sterilization of the coupons was performed for
30 min. Abiotic control tests were conducted under identical conditions [19].

2.3. Electrochemical Measurements

Electrochemical tests were performed using an electrochemical workstation (CHI
660D, CH Instruments, Austin, TX, USA). The tests included open circuit potential (OCP),
electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization curves. A
platinum plate and a saturated calomel electrode (SCE) served as the counter and reference
electrodes, respectively. Coupons with a 1 cm² surface exposed were utilized as the working
electrode. EIS measurements were conducted at stable OCP conditions, exciting the system
with a sinusoidal signal of 10 mV amplitude, over frequencies ranging from 0.01 Hz to
100 kHz, on days 1, 5, and 8. EIS data were analyzed to derive parameters and equivalent
electrical circuits using ZSimDemo software (Version 3.30d, EChem Software, Ann Arbor,
MI, USA). Corrosion potential (Ecorr), corrosion current density (Icorr), anodic slope (βa),
and cathodic slope (βc) were determined from the potentiodynamic polarization results.
These tests were carried out after an 8-day incubation period, with scanning potentials
from −0.5 V to +0.5 V relative to OCP at a rate of 1 mV/s.

2.4. Surface Characterizations and Corrosion Product Analyses

The surface morphologies of the coupons from both bacterial and abiotic tests were
examined using scanning electron microscopy (SEM, FEI Quanta 200F, Hillsboro, OR,
USA) after 8 days of incubation. For biofilm fixation, the coupons were immersed in
2.5% glutaraldehyde for 30 min. A graded dehydration process followed, using ethanol
concentrations of 25%, 50%, 75%, and 100%, with each step lasting 10 min. Pit morphology
was analyzed after removing corrosion products with a freshly prepared Clarke solution
(20 g Sb₂O₃, 50 g SnCl₂ in concentrated hydrochloric acid to make 1000 mL) [36], and pit
depths were measured using confocal laser scanning microscopy (CLSM, OLS4100-SAF,
OLYMPUS, Tokyo, Japan). To determine the valence states of corrosion products and
elemental compositions, energy-dispersive X-ray spectroscopy (EDS, FEI Quanta 200F,
Hillsboro, OR, USA) and X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Fisher,
Waltham, MA, USA) were used. The indexed peaks were calibrated using the C 1s peak at
a binding energy of 284.8 eV.

2.5. Weight Loss Measurement

The general corrosion rate of the coupons after an 8-day incubation at 38 °C was
determined by the weight loss method [37]. The weights of the coupons were recorded
both before the incubation using an analytical balance (minimum unit 0.1 mg). After
incubation, the corrosion products were cleaned using Clark’s solution, followed by the
standard procedures for preparing corrosion specimens. The exposed surfaces were rinsed
with deionized water, cleaned with pure alcohol, and dried using a high-purity nitrogen
gas stream. Subsequently, the weights of the coupons were measured again to determine
the corrosion rate. The corrosion rate was calculated as the following Equation (1):

\[
\text{Corrosion Rate} = \frac{(K \times W)}{(A \times T \times D)}
\]

where \(K\) is a constant number 87,600, \(W\) is the weight loss in g, \(A\) is the surface area in cm²,
\(T\) is the test duration in hours, and \(D\) is the density of samples in g/cm³.
3. Results

3.1. Weight Loss

The influence of symbiotic SRB and TGB on general corrosion was assessed by measuring the weight loss of steel coupons. As depicted in Figure 1, after an 8-day incubation at 38 °C in various culture media, coupons exposed to microbial environments exhibited significantly higher weight loss compared to those in abiotic control media, indicating corrosion facilitated by microorganisms. Notably, the weight loss observed in the SRB medium was more than tenfold higher than that in the TGB medium. Furthermore, the system containing a mixture of SRB and TGB showed the highest weight loss, exceeding that in the SRB-only medium by more than 1.75 times and in the TGB-only medium by 18 times. These results suggest that microbiologically influenced corrosion (MIC) occurs in systems containing these microorganisms, with the mixed culture of SRB and TGB substantially accelerating the corrosion process.

![Figure 1](image1.png)

**Figure 1.** Weight loss of coupons for 8-day incubation with or without bacteria.

3.2. Morphological Study of Corroded Samples

Figure 2 illustrates the SEM morphologies of biofilms (or corrosion products) formed after an 8-day incubation at 38 °C in various microbial systems. In Figure 2A,B, massive porous biofilms are visible on the surfaces of the coupons, indicative of the strong metabolic activity of the cells and significant corrosion due to microbiologically influenced corrosion (MIC) in the SRB-containing and mixed SRB-TGB systems. High-resolution SEM images (Figure 2A',B') reveal different shapes of SRB and TGB cells embedded in the exocellular polymeric substance (EPS). A thinner biofilm layer is noted on the surfaces of coupons in Figure 2C, suggesting less severe corrosion in the TGB-only system. This observation is supported by the appearance of thin and dense biofilms in the high-resolution SEM image (Figure 2C'). The SEM findings correlate well with the weight loss data previously discussed.
Figure 2. SEM morphologies of biofilms (or corrosion products) for 8-day incubation with bacteria: (A,A') SRB; (B,B') SRB + TGB; and (C,C') TGB.

Figure 3 displays the SEM morphologies of the coupon surfaces after the removal of biofilms (or corrosion products) following the same 8-day incubation period. In the SRB-only system, the rough coupon surface and presence of pits, shown in Figure 3A, suggest pronounced MIC caused by SRB. The most severe surface roughness and pitting occur in the mixed SRB and TGB system, as evident in Figure 3B, indicating that the symbiosis significantly exacerbates MIC. In contrast, Figure 3C shows a smoother coupon surface with visible polish lines in the TGB-only system, indicative of typical localized corrosion beneath the TGB biofilm, which aligns with the observed slight MIC and is consistent with the earlier weight loss data.
Figure 3. SEM morphologies of coupon surfaces after corrosion products or biofilm removal for 8-day incubation with bacteria: (A) SRB; (B) SRB + TGB; and (C) TGB.

Figure 4 displays the pit morphologies of coupon surfaces after the removal of biofilms (or corrosion products) following an 8-day incubation at 38 °C. Smooth surfaces are evident on coupons immersed in the control systems (Figure 4a–c), whereas obvious pits are observed on coupons immersed in microorganism-containing systems (Figure 4a’–c’), indicating a significant influence of SRB and TGB on the corrosion process. Notably, the most severe MIC pitting and irregular pits are observed on coupons immersed in the mixed SRB and TGB system.

Figure 5 presents CLSM images of pit profiles. The wavy surfaces seen in Figure 5a–c confirm that coupons in the control systems experience slight corrosion, consistent with the findings from pit morphologies (Figure 4a–c). In contrast, wide and deep pits are formed in the microorganism-containing systems (Figure 5a’–c’), indicating the occurrence of MIC pitting. The maximum pit depth detected is 31.75 µm in the mixed SRB and TGB system, compared to 31.38 µm in the SRB-containing system and 14.61 µm in the TGB-containing system. These deeper and wider pits in the mixed SRB and TGB system suggest that the combined culture of SRB and TGB leads to more severe MIC pitting. The depths of the pits are listed in Table 1 for reference.

Table 1. Pitting depth for coupon surfaces after corrosion products or biofilm removal for 8-day incubation with or without bacteria.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control SRB</th>
<th>Control SRB + TGB</th>
<th>Control TGB</th>
<th>SRB</th>
<th>SRB + TGB</th>
<th>TGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (µm)</td>
<td>10.44</td>
<td>10.02</td>
<td>8.70</td>
<td>31.38</td>
<td>31.75</td>
<td>14.61</td>
</tr>
</tbody>
</table>
Figure 4. Morphologies for coupon surfaces after corrosion products or biofilm removal for 8 day-incubation with or without bacteria: (a) control SRB, (b) control SRB + TGB, (c) control TGB, (a') SRB, (b') SRB + TGB, and (c') TGB.

Figure 5. Pit profiles for coupon surfaces after corrosion products or biofilm removal for 8-day incubation with or without bacteria: (a) control SRB, (b) control SRB + TGB, (c) control TGB, (a') SRB, (b') SRB + TGB, and (c') TGB.
3.3. EDS and XPS Study of Corrosion Products

Substance information was analyzed using X-ray photoelectron spectroscopy (XPS) and energy-dispersive X-ray spectroscopy (EDS). The EDS findings corroborate the results obtained from XPS analysis. Figure 6 illustrates the EDS analysis of coupons after an 8-day incubation in microorganism-containing systems at 38 °C. Abundant carbon, oxygen, and iron elements are detected in all systems, confirming the presence of iron oxides and organic substances such as nucleic acids, proteins, polypeptides, and enzymes in the biofilms. Sulfur elements are detected in the SRB-containing system, while trace sulfur is found on coupons in the TGB-containing system, indicating that FeS is the primary corrosion product of MIC caused by SRB.

![Figure 6](image_url)

Table 1. Substance information was analyzed using X-ray photoelectron spectroscopy (XPS) and energy-dispersive X-ray spectroscopy (EDS). The EDS findings corroborate the results obtained from XPS analysis. Figure 7 presents a high-resolution XPS spectra of C 1s, N 1s, O 1s, S 2p, and Fe 2p after the 8-day incubation in microorganism-containing systems at 38 °C. In the C 1s spectra, peaks at 286.5 and 288.7 eV correspond to -COOH, and peaks at 283.6 and 285.8 eV correspond to Fe 3C and C-O, respectively. In the N 1s spectra, peaks at 398.8 and 400.6 eV correspond to -NH 2. The O 1s spectra reveal peaks at 530.9 and 531.9 eV corresponding to -COOH and peaks at 529.8 and 532.0 eV corresponding to Fe 2O3, with additional peaks at 529.7 and 533.2 eV corresponding to FeOOH and -OH, respectively. The S 2p spectra display peaks at 161.4 eV corresponding to FeS and peaks at 163.4 and 169.9 eV corresponding to HSCH 2− and SO 4 2−. The Fe 2p spectra show peaks at 710.3 and 724.3 eV corresponding to FeS and FeOOH and peaks at 711.3, 712.5, and 725.7 eV corresponding to Fe 2O3 and Fe 3+ . Chemical bonds such as -COOH and -OH are components of nucleic acids and cell walls, while -NH 2 and HSCH 2− are components of amino acids forming proteins, polypeptides, and enzymes. The main corrosion products identified are FeOOH and FeS in the SRB-containing system, Fe 2O3 in the TGB-containing system, and a combination of Fe 2O3, FeOOH, and FeS in the mixed SRB + TGB system, suggesting that both SRB and TGB contribute to MIC simultaneously.
The Fe 2p spectra show peaks at 710.3 and 724.3 eV corresponding to FeS and FeOOH and peaks at 711.3, 712.5, and 725.7 eV corresponding to Fe$_2$O$_3$ and Fe$^{3+}$. Chemical bonds such as -COOH and -OH are components of nucleic acids and cell walls, while -NH$_2$ and HSCH$_2$- are components of amino acids forming proteins, polypeptides, and enzymes.

The main corrosion products identified are FeOOH and FeS in the SRB-containing system, Fe$_2$O$_3$ in the TGB-containing system, and a combination of Fe$_2$O$_3$, FeOOH, and FeS in the mixed SRB + TGB system, suggesting that both SRB and TGB contribute to MIC simultaneously.

Figure 7. XPS analysis of corrosion products or biofilm for 8-day incubation with bacteria: (a) SRB; (b) SRB + TGB; and (c) TGB.
3.4. Electrochemical Measurements

Microorganisms exert significant effects on the electrochemical behavior of carbon steel, while the electrochemical responses from biofilms reflect microbial activities. Figure 8 displays the open circuit potential (OCP) values measured at the end of the 1st, 3rd, 5th, and 8th days during an 8-day incubation period in microorganism-containing systems at 38 °C. The OCP values exhibit an increasing trend in all microorganism-containing systems, indicating the formation of the protective films of corrosion products on coupon surfaces. However, the OCP values sharply decrease to approximately −894 and −866 mV at the end of the 8th day in the SRB-containing system and mixed system, respectively, suggesting the formation of corrosive and porous biofilms on coupon surfaces, as depicted in Figure 2A,B.

![Figure 8](image_url)  
Figure 8. Changes in OCP versus time during the 8-day incubation with bacteria.

Figure 9 illustrates the Nyquist and Bode plots obtained from electrochemical impedance spectroscopy (EIS) measurements conducted at the end of the 1st, 3rd, and 8th days during an 8-day incubation period in microorganism-containing systems at 38 °C. The diameter of the Nyquist plot semi-circle indicates the resistance to MIC attack and the corrosion rate. Initially, the coupons exhibit the largest Nyquist plot semi-circle diameter in all microorganism-containing systems on the 1st day, which then decreases over time, except for a slight increasing trend observed on the 8th day in the TGB-containing system. This trend suggests the precipitation of the protective films of corrosion products on coupon surfaces during the initial stage, followed by the formation of corrosive biofilms as bacteria adsorb onto the surfaces during the incubation period, as depicted in Figure 2A,B.

Moreover, in the TGB-containing system, the EIS curves exhibit inductive reactance in the low-frequency range. Neville et al. identified this phenomenon as resulting from the dynamic process of the adsorption–desorption–adsorption of corrosion inhibitors [38]. The inductive reactance arises from the adsorption–desorption–adsorption process of macromolecular substances such as peptone and beef extract. The impedance trends observed in the Bode plots align with those in the Nyquist plots. The electrochemical parameters and equivalent circuits obtained from fitting are presented in Table 2 and Figure 10. The symbols $R_s$, $R_f$, and $R_{ct}$ represent the solution resistance, the resistance of the biofilm and corrosion product layer, and the charge transfer resistance, respectively. $Q_f$ and $Q_{dl}$ denote the capacitance of the biofilm and corrosion product film and the double-layer capacitance, respectively, while L stands for inductive reactance.
Figure 9. Nyquist and Bode plots for coupons at end of 1st, 3rd, and 8th day during 8-day incubation test period in 500 mL electrochemical cells with different bacteria: (A,A’) SRB; (B,B’) SRB + TGB; and (C,C’) TGB.

Table 2. EIS-derived electrochemical parameters of coupons at end of 1st, 3rd, and 8th day during 8-day incubation test period in 500 mL electrochemical cells with bacteria.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day</th>
<th>( R_s ) (Ω cm(^2))</th>
<th>( R_f ) (Ω cm(^2))</th>
<th>( R_{ct} ) (Ω cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>1</td>
<td>5.5</td>
<td>4.0</td>
<td>2898</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.7</td>
<td>5.7</td>
<td>3369</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5.9</td>
<td>27.7</td>
<td>4889</td>
</tr>
<tr>
<td>SRB + TGB</td>
<td>1</td>
<td>18.3</td>
<td>-</td>
<td>6495</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.1</td>
<td>161.8</td>
<td>814</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>12.6</td>
<td>41.8</td>
<td>3203</td>
</tr>
<tr>
<td>TGB</td>
<td>1</td>
<td>154.1</td>
<td>-</td>
<td>8127</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>166.9</td>
<td>422.6</td>
<td>563</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>170.3</td>
<td>540.7</td>
<td>1109</td>
</tr>
</tbody>
</table>
In Table 2, the $R_f$ value in the TGB-containing system exceeds that of the other systems, indicating the formation of dense biofilms and corrosion products on the carbon steel surface. These dense biofilms and corrosion products create an anaerobic environment favorable for SRB growth, promoting electron transfer from iron. Conversely, the $R_f$ values in the SRB-containing system are smaller, suggesting weak protection from porous biofilms and corrosion products on the carbon steel surface. The $R_{ct}$ value in the TGB-containing system and mixed system decreases during the initial stage and increases at the end of the MIC stage, indicating an increase in MIC caused by TGB as biofilms form on the carbon steel surface, followed by a decrease in bacterial activity as $O_2$ and nutrient substances such as peptone and beef extract are consumed.

Interestingly, the $R_{ct}$ value in the SRB-containing system increases during the incubation period, potentially related to the extracellular electron transfer (EET) process in MIC caused by SRB. The results of $R_{ct}+R_f$, shown in Figure 11, exhibit the same trends as the $R_{ct}$ results. In the SRB-containing system and mixed system, the values of double-layer capacitance $Q_{dl}$ increase during the incubation period, indicating the formation of highly conductive corrosion products, such as iron sulfide [39].

Figure 12 presents the potentiodynamic polarization curves obtained at the end of the 8th day after an 8-day incubation in microorganism-containing systems at 38 °C. The fitting parameters obtained from the Tafel curve analysis are summarized in Table 3. Significantly higher corrosion potential and lower corrosion current density are observed in the TGB-containing system.

Table 3. Electrochemical parameters calculated from potentiodynamic polarization curves obtained at the end of the 8-day incubation.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$E_{corr}$ (mV) vs. SCE</th>
<th>$i_{corr}$ (μA cm$^{-2}$)</th>
<th>$\beta_a$ (mV dec$^{-1}$)</th>
<th>$\beta_c$ (mV dec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>−763</td>
<td>14.5</td>
<td>114</td>
<td>−59</td>
</tr>
<tr>
<td>SRB + TGB</td>
<td>−779</td>
<td>21.1</td>
<td>126</td>
<td>−140</td>
</tr>
<tr>
<td>TGB</td>
<td>−562</td>
<td>3.1</td>
<td>57</td>
<td>−162</td>
</tr>
</tbody>
</table>
capacitance $Q_{dl}$ increase during the incubation period, indicating the formation of highly conductive corrosion products, such as iron sulfide [39].

Figure 11. Time-dependent changes in $R_f + R_{ct}$ for coupons at end of 1st, 3rd, and 8th day during 8-day incubation test period in 500 mL electrochemical cells with bacteria.

Figure 12 presents the potentiodynamic polarization curves obtained at the end of the 8th day after an 8-day incubation in microorganism-containing systems at 38 °C. The fitting parameters obtained from the Tafel curve analysis are summarized in Table 3. Significantly higher corrosion potential and lower corrosion current density are observed in the TGB-containing system.

4. Discussions

The weight loss data across all systems with microbes are higher than those in control systems, which is attributed to microbiologically influenced corrosion (MIC) caused by SRB and TGB. According to Figure 1, MIC due to TGB is considerably less severe compared to that caused by SRB, indicating that SRB are the predominant microorganisms leading to MIC. Furthermore, the symbiosis of TGB and SRB results in the most severe MIC, more than
1.75 times that of the SRB-containing system and 18 times that of the TGB-containing system, as shown in Figure 1. This suggests that the symbiosis between TGB and SRB enhances MIC. The pitting data from Figure 5 and Table 1, along with the corrosion morphology in Figure 4, corroborate the weight loss data. In control systems, uniform corrosion is the primary type observed, whereas pitting attack occurs in all microorganism-containing systems. The symbiosis of SRB and TGB significantly intensifies pitting attacks, with the deepest pitting depths observed in the mixed system of SRB and TGB, as detailed in Table 1. These findings confirm that the symbiosis of SRB and TGB alters the conventional corrosion process, accelerating pitting attacks. The mechanism of MIC resulting from this symbiosis will be discussed further.

Generally, MIC by SRB is initiated due to the bioenergetic requirements of SRB [40] and is typically categorized into extracellular electron transfer (EET)-MIC [19,26]. It has been demonstrated that the EET process is a crucial step in MIC at the genetic level and represents a limitation of the MIC process [41]. MIC caused by SRB produces the main corrosion product, FeS (Figure 7), which acts as a semiconductor providing an electron transfer pathway for the EET process between the coupon surface and sessile cells [42], thus not inhibiting the MIC attack. It has been revealed that a complete FeS layer fails to prevent MIC pitting [42]. Additionally, porous biofilms (Figure 2A,B) on the surface of coupons can facilitate the transfer of enzymes and mediators [43], which involve mediated electron transfer (MET), another mechanism for cells to harvest electrons. Conversely, the dense biofilms caused by TGB (Figure 2C) are not porous, hindering the transfer of nutrients, oxygen, enzymes, and mediators essential for TGB cells, resulting in only slight MIC. Furthermore, in the later stages of incubation, the consumption of nutrients and oxygen may also lead to the diminished bioactivity of TGB cells. Conversely, dense biofilms (Figure 2 and Table 2) provide a better anaerobic environment for the growth of SRB in the mixed SRB and TGB system [42], explaining the severe MIC resulting from the symbiosis of SRB and TGB (Figure 4).

Biofilms formed on the surface of metals alter the physical and chemical properties at the interface between the bulk solution and the metal, significantly impacting the corrosion behavior of the substrate. Figure 8 shows that the open circuit potential (OCP) values increase from the first to the fifth day, suggesting that organic components in the culture media are adsorbed, and biofilms are formed on the surface. However, in the TGB-containing system, the consumption of O$_2$ results in reduced microbial activity and the formation of dense biofilms and Fe$_2$O$_3$ corrosion products, as illustrated in Figures 2C and 6c, leading to higher OCP values in the later period.

Conversely, in the SRB-containing system and the mixed system, the reduction in nutrients and the formation of complete biofilms and FeS on the surface, as shown in Figure 2A,B and Figure 6a,b, result in the use of the Fe element as an electron donor for the reduction of sulfate, leading to lower OCP values in the later period. A lower OCP value indicates a higher tendency for corrosion. Interestingly, the $R_d + R_{ct}$ values of coupons in the TGB-containing system are lower than those in the SRB-containing and mixed systems during the incubation period, except on the first day, as depicted in Figure 11. This discrepancy may be due to the fact that external electron mediators, self-secreted cytochromes, and enzymes, which accelerate electron transfer in the microbial community, are not reflected in the $R_{ct}$ values of the electrochemical response.

Different microbes result in varying metabolisms, impacting the types of corrosion products formed. From the XPS analysis presented in Figure 7, FeS is identified as the main corrosion product in MIC by SRB, observed on the surface in Figure 7a. The mechanism of MIC by SRB can be introduced as follows [40]:

Anodic reaction:

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

Cathodic reaction:

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

\[ \text{Fe}^{2+} + \text{HS}^- \rightarrow \text{FeS} + \text{H}^+ \]  \hspace{1cm} (4)

\[ \text{HS}^- + \text{H}^+ \rightarrow \text{H}_2\text{S} \] \hspace{1cm} (5)

It has been demonstrated that the precipitation of iron sulfide and the formation of hydrogen sulfide promote the development of pits \cite{18,42}. Furthermore, corrosive \( \text{H}_2\text{S} \) produced by SRB under biofilms leads to local acidification, which facilitates pitting corrosion \cite{44,45}.

TGB, being aerobic bacteria, might generate energy through the oxidation of Fe or \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \), using \( \text{O}_2 \) as the electron acceptor. The dense biofilms and corrosion products, such as \( \text{Fe}_2\text{O}_3 \) produced by TGB and depicted in Figure 2C, deposit on the coupon surface, inducing MIC, particularly pitting corrosion.

Anodic reaction:

\[ \text{Fe} \xrightarrow{\text{biocatalyst}} \text{Fe}^{2+} + 2\text{e}^- \] \hspace{1cm} (6)

\[ \text{Fe}^{2+} \xrightarrow{\text{biocatalyst}} \text{Fe}^{3+} + \text{e}^- \] \hspace{1cm} (7)

Cathodic reaction:

\[ \text{O}_2 + 2\text{H}_2\text{O} + 4\text{e}^- \rightarrow 4\text{OH}^- \] \hspace{1cm} (8)

The reactions for corrosion products are as follows:

\[ \text{Fe}^{2+} + 2\text{OH}^- \rightarrow \text{Fe(OH)}_2 \] \hspace{1cm} (9)

\[ 4\text{Fe(OH)}_2 + \text{O}_2 \rightarrow 4\text{FeOOH} + 2\text{H}_2\text{O} \] \hspace{1cm} (10)

\[ 2\text{FeOOH} \rightarrow \text{Fe}_2\text{O}_3 + \text{H}_2\text{O} \] \hspace{1cm} (11)

\[ \text{Fe}^{3+} + 3\text{OH}^- \rightarrow \text{Fe(OH)}_3 \] \hspace{1cm} (12)

\[ 2\text{Fe(OH)}_3 \rightarrow \text{Fe}_2\text{O}_3 + 3\text{H}_2\text{O} \] \hspace{1cm} (13)

Thus, the presence of \( \text{FeS}, \text{Fe}_2\text{O}_3 \), and \( \text{FeOOH} \) in the mixed system of SRB and TGB, as shown in Figure 7b, is expected. SRB cannot thrive but merely survive in an oxygen-containing system \cite{42}. Due to the oxygen presence in the TGB medium, SRB are exposed to an oxygenated environment in the mixed system of SRB and TGB. Nonetheless, the symbiosis of TGB and SRB leads to severe MIC, underscoring the significant role of TGB in this process. The addition of TGB accelerates the consumption of Fe and \( \text{Fe}^{2+} \) in Reactions (6) and (7), promoting Reaction (2) and resulting in more dissolved Fe substrate. Subsequently, increased FeS formation on the coupon surface in Reaction (4) provides additional attachment sites for SRB and TGB, as evidenced in Figures 6 and 7. King and Miller \cite{46} reported that areas covered by SRB biofilms serve as cathodic sites, while areas not covered by biofilms act as anodic sites. Therefore, the FeS film on the coupon surface provides additional cathodic spots, enabling more SRB and TGB to attach and harvest more electrons from the Fe substrate, thus accelerating Reaction (3), which enhances EET-MIC and pitting corrosion. It has been shown that oxygen levels drop to zero beneath tubercle layers, creating an ideal environment for anaerobes \cite{47,48}. Consequently, the dense biofilms and corrosion products produced by TGB, covering the coupon surface as shown in Figure 2C and Table 2, may form outer films that provide a locally oxygen-free shelter conducive to the enhanced growth of SRB, leading to severe pitting corrosion.

5. Conclusions

This investigation aims to further discuss the impacts of bacterial symbiosis on MIC in carbon steel. The presence of sulfate-reducing bacteria (SRB) alone resulted in severe extra-cellular electron transfer (EET)-MIC and MIC pitting, whereas the presence of total general bacteria (TGB) alone led to only slight MIC. SRB is identified as the primary corrosive agent. In the mixed culture of SRB and TGB, MIC and MIC pitting were notably severe due to the dense biofilms and corrosion products produced by TGB. These form outer films, providing
a locally anaerobic environment conducive to SRB growth and the formation of iron sulfide (FeS), which serves as an electron transfer pathway. Increased FeS provides additional areas for bacterial attachment, facilitating more extensive electron transfer. The main corrosion products in the mixed system of SRB and TGB were FeS, iron(III) oxide (Fe_2O_3), and iron oxyhydroxide (FeOOH). Based on these results, the symbiosis of bacteria plays a significant and complex role in the corrosion behaviors of carbon steel, thus warranting consideration in discussions of MIC for metals. Furthermore, the limitations and effects of electrochemical measurements on MIC should also be considered, given the sensitivity and specificity of microorganisms in corrosion processes.

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