Biological metal corrosion in saline systems by sulfur-reducing and iron-oxidizing bacteria

Eun-Hae Sung, Ji-Sun Han, Chang-Min Ahn, Hyung Joon Seo and Chang-Gyun Kim

ABSTRACT

This study investigated whether any possible bio-corrosion of pumps could occur when operating underground pumping stations in coastal regions. Groundwater in the stations was found to contain *Leptothrix* sp. (iron-oxidizing bacteria, IOB) and *Desulfovibrio* sp. (sulfur-reducing bacteria, SRB). Four different metal specimens were exposed to saline water media, where *Leptothrix* sp. or *Desulfovibrio* sp. were inoculated solely or together. The result showed that IOB not only provoke the corrosion of galvanized and stainless steels but also accelerate (by 5–10 times) the formation of zinc/iron precipitates. The SRB specifically mediated the corrosion of zinc steel to a greater extent than the IOB. In a single medium, STS 304, galvanized steel, iron and zinc steel resisted corrosion in that order. However, in the mixed culture, the metals were corroded by a factor of 2–7 more than in the single medium. On disinfection, a higher NaOCl concentration surprisingly caused increased chemical corrosion, and UV light scattering due to corrosion precipitates enhanced microbial corrosion. Consequently, the metals showed more biochemical corrosion than the control, especially in a mixed culture. In particular, the level of STS 304 corrosion was significantly higher in the presence of microbes than the control.

Key words | iron corrosion, iron-oxidizing bacteria, sulfur-reducing bacteria

INTRODUCTION

Underground constructions located beneath and within a transitional zone in coastal regions are often vulnerable to breakdown, damage or corrosion due to retentive contact with saline water, which intrudes toward a coastal aquifer. In particular, utilities at pumping stations that were initially designed and built with anti-corrosion properties against seawater intrusion are exposed to corrosive environments, which impairs the pumps and pipelines due to metal oxide blockages and scale deposition (Korea Gas Corporation R&D Division 2004). The growth of iron-utilizing bacteria can result in drinking water with a metallic and bitter taste that can become yellowish due to iron corrosion precipitates, which can in turn break down the transport system (Korea Gas Corporation R&D Division 2004; Xu et al. 2007, 2008). To cope with these problems, pumping stations and pipelines should be managed and controlled. In coastal regions, anti-corrosive materials, such as stainless steel or copper, have been used in the manufacture of pumps, but they become severely pitted, creviced and cracked, producing deposits and precipitates (Ministry of Agriculture and Forestry 1997). This is closely related to galvanic corrosion and passivation, but can be associated simultaneously with a microbial metabolism under specific conditions (David & Niels 1994). In general, iron-oxidizing bacteria (IOB) oxidize iron to the trivalent form, where the electrons released are used as energy sources for their metabolism. Iron hydroxide complexes subsequently settle, which can cause a blockage in the pipeline transport system. This can occur persistently under the influence of microbial metabolism. The dissolved iron can also be
transformed into a jelly like slime, which can clog the pipeline. Sulfur-reducing bacteria (SRB) commonly attack metals under anaerobic conditions, which produce hydrogen sulfide and cause sulfide stress cracking of the metal as a biological corrosion stimulant (Ministry of Agriculture and Forestry 1997).

In the present study, saline groundwater samples were taken from designated pumping wells at the pumping station, which was in hydraulic continuity with seawater intrusion within a southern coastal zone, Incheon, Korea. The pumps were visibly corroded, resulting in precipitates cumulatively clogging the pipeline system, even when the equipment was composed of corrosive-resistant materials, i.e. stainless steel. Accordingly, microbial corrosion of various metal coupons (iron, galvanized, stainless and zinc steels) using IOB and SRB was investigated. 16S rDNA analyses of the site groundwater samples were carried out to determine the microbial diversity and dominant species that induce severe corrosion (e.g. IOB and SRB). Minimal media supplemented with these dominant species were incubated with four different metal coupons (iron, galvanized steel, zinc and stainless steel (STS304)) under a range of conditions. The effects of microbial growth on metal corrosion-associated precipitation were evaluated. Disinfection with either NaOCl addition or UV illumination exposure was implemented to effectively deactivate microbial growth limiting the bio-corrosion of metals.

MATERIALS AND METHODS

Groundwater sampling from pumping well

In February 2007, a total of four groundwater samples which were under tidal influence in a coastal zone at Incheon, Korea, were each taken from four pumping wells using a sanitized baler. The bores were initially installed to evacuate saline water and prevent the underground facilities from corrosion. Nevertheless, the pumps and their connections were corroded more severely than expected within a shorter time frame, even though they had been constructed using anti-corrosion materials. This resulted in the early replacement of pumps, which cost $100,000 each, totaling $400,000 for the four wells every 6 months. The pumps in the wells were located at B-5, B-12, B-15 and B-16, where saline water had been pumped out.

Before taking groundwater samples, the wells had been previously purged with 1 l of bailer (Cole-Parmer, USA) to the equivalent of two to three volumes of the screen intervals. The groundwater samples were collected into 1 l sterilized sampling bags, stored at 4 °C and transported to the laboratory. The dissolved oxygen (DO), oxidation-reduction potential (ORP) and electric conductivity (EC) were observed at sampling locations using a DO meter (YSI 550A Portable DO Meter, USA), ORP meter (Orion 230A Portable ORP Meter, USA) and conductivity meter (YSI, Model 30, USA), respectively.

The concentrations of inorganic compounds, such as SO$_4^{2−}$, Cl$^−$, NO$_2$ and NO$_3$ were determined by ion chromatography (Yong Lin Instrument, Water 432 Conductivity Detector, Korea) after filtering the samples through a 0.45 µm membrane filter (Whatman, Cat. No. 6786 2502). The concentrations of dissolved iron and zinc were also determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Varian, Liberty Series 2, USA) after acidifying the samples with 1 M HCl.

Microbial analysis

A 200 ml groundwater sample was centrifuged for 15 min at 5,000 rpm (HA-1000-3, Hanil Science, Korea). The genomic DNA possibly retained in the concentrated solids (~500 mg) was effectively retrieved for 5 s at speed 5 on a FastPrep® Instrument (Bio101 system, Q-Bio gene, USA) with the total genomic DNA, and extracted using a FastDNA® SPIN kit (Bio101 system, Q-Bio gene, USA).

To survey the diversity of the microbial species in the sample, 16S rDNA was amplified selectively using the universal primer, 27F (5’ AGA GTT TGA TCM TGG CTC AG 3’) and 1492R (5’ TAC GGY TAC CTT GTT ACG ACT T 3’) (Sungur et al. 2007). Denaturation (94 °C), annealing (56 °C) and polymerization (72 °C) were carried out sequentially for a total of 35 cycles. The amplified PCR product was then verified in 0.8% agarose gel by electrophoresis for 30 min at 100 mV (Mupid-α, Japan). The resulting 16S rDNA gel was purified using both Winzard® SV Gel and PCR Clean-Up System (Promega, USA). The refined 16S rDNA was ligated to a pGEM-T easy vector (Promega,
USA), and transformed into competent cells (Escherichia coli XL1-blue). The cells were incubated selectively on LB (Luria-Bertani) media that had been previously treated with ampicillin and X-gal/IPTG (Promega, USA). The plasmids in the competent cells were finally purified using a Winzard® Plus Minipreps DNA Purification System (Promega, USA). The DNA sequences were characterized using the purified plasmid on a 3,100 Capillary DNA Sequencer (Applied Biosystems, USA). The analyzed DNA sequences were identified comparably by the 16S rDNA sequences stored in the BLAST Network Service, NCBI (National Center for Biotechnology Information, USA). The microbial diversity along with the dominant species from the sample was determined by referring to a frequency of occurrence coinciding physiology >98%.

**Metal coupon preparation and corrosion experiment**

Four types of metal coupon (iron, galvanized steel, zinc and stainless steel (STS304)) were used in the tests. Each coupon with a nominal size of 8 × 2 cm were cleaned by sonification for 60 min (BRANSONIC 5510R-DTH, Mexico), and subsequently used for a set of corrosion tests. The material of the sump pumps operating at the pumping station in the studied area was STS 304. The other three materials were chosen to compare the corrosion properties with those of STS 304. The physical properties of the four different coupons were analyzed by X-ray fluorescence spectroscopy (XRF, Axios, Phillips, The Netherlands).

The corrosion tests on the four different metal coupons were performed in nutrient-rich medium in 0.01 M NaCl inoculated with the microorganism of concern. The control was not inoculated. IOB and SRB were inoculated individually or together.

A control was prepared for each type of coupon such that quintuple coupons were initially placed in a 2 l round-bottom Pyrex flask filled with 1.5 l of nutrient-rich medium, which was then closed airtight with a Teflon-lined silicon-stopper. The flasks were then placed in an incubator (30 ± 0.5 °C) and the corrosion test was started (VISION Scientific Co., Korea). A 100 ml sample was taken from the test flask every two days, which was quickly replaced with the same volume of nutrient-rich medium. The metal concentrations (i.e., iron, zinc), pH, DO, ORP and EC were obtained. In addition, the total iron and zinc concentrations were determined using EPA Method 3015A. An aqueous phase sample was extracted using a microwave extractor (MARS X Microwave, CEM Corporation, USA), and then filtered through a 0.45 μm membrane filter. The filtrate was acidified with 0.24 M HCl. The metal concentrations in the filtrate were quantified by ICP-OES. The rust products released into the nutrient-rich medium during the test were characterized by X-ray diffraction (XRD, Rigaku DMAX 2500, Japan) and XRF.

**Disinfection test**

Disinfection tests were performed using either NaOCl addition or UV illumination to limit biological metal oxidation (Park 2004; Kim et al. 2006). Disinfection tests were carried out against the microorganisms of concern in the presence of two different metal coupons (iron and STS304) being dipped into the nutrient-rich medium. For the control, one piece of a coupon (20 × 80 mm) was placed in a 500 ml round-bottom Pyrex flask filled with 300 ml of nutrient-rich medium and closed airtight with a Teflon-lined silicone-stopper. The test sample was inoculated with microorganisms that had previously been adapted for 2 days at 30 °C in an incubator (VISION Scientific Co., Korea).

After a two day adaptation period, deactivating tests were initiated by diluting 1,000 ppmv of a NaOCl stock solution to 2, 20 and 200 ppmv into the test flasks. The tests were carried out for 7 days. During the test period, 100 ml of the sample was taken every two days, and replaced immediately with the same volume of medium. Subsequently, the total and dissolved iron concentrations in the sample were determined. Incubation of the sample for the microbial viability was terminated on day 7. At this time, the microbial population remaining in the sample was counted.

For the UV irradiation disinfection test, UV light (SANKYO DENKI G30T8) at 253.7 nm with a power intensity of 1,032 μW cm⁻² was illuminated from a source hanging on the ceiling 30 cm above the test flask placed on the bottom of a clean bench (Han-dock Biotech, Korea). Samples were taken at the initial stage, as well as on the first, second and third days from the start of the test, and continued every two days until the seventh day. The total and
dissolved iron concentrations in the sample were then observed and the microbial population was also counted.

**Preparation of the media and microbial cultivation**

Corrosion tests were carried out in nutrient-rich medium, amended with 4.12 g MgSO₄·7H₂O, 5 g sodium citrate, 1.26 g CaSO₄·2H₂O, 1.0 g NH₄Cl, 1.0 g K₂HPO₄, 3.5 g sodium lactate and 1.0 g yeast extract dissolved in 1 l of sterilized double distilled water (Xu et al. 2008). To simulate the salinity of the groundwater samples in the laboratory, NaCl (98%, Dongyang Chem.) was added to the nutrient-rich medium to similar levels observed in the pumping wells at the site (approximately 0.01 M NaCl). The pH was adjusted to 7.5 using 2 M NaOH. The solutions were then sterilized at 121°C for 20 min (MLS-3780 SANYO, Japan).

The microbes causing metal corrosion found specifically in this study, i.e. *Leptothrix mobilis* (DSMZ 10617) as IOB and *Desulfovibrio aespoeensis* (DSMZ 10631) as SRB, were purchased from DSME (Germany). To cultivate the IOB, Sphaerotilus-Leptothrix medium (1.00 g yeast extract, 1.50 g peptone, 0.20 g MgSO₄·7H₂O, 0.05 g CaCl₂, 0.50 g ferric ammonium citrate, 0.05 g MnSO₄·H₂O and 0.01 g FeCl₃·6H₂O) was prepared in 1 l of double distilled water, which was then adjusted to pH 7.2 with 1 M NaOH. The solution was finally sterilized at 121°C for 20 min (MLS-3780 SANYO, Japan). The medium was inoculated with IOB, mixed at 200 rpm for 2 days at 30°C under aerobic conditions (VISION Scientific Co., Korea), and used for the metal corrosion test along with a control.

Postgate C medium was prepared for cultivating SRB (0.5 g KH₂PO₄, 1.0 g NH₄Cl, 4.5 g Na₂SO₄, 0.06 g CaCl₂·2H₂O, 0.06 g MgSO₄·7H₂O, 6.0 g sodium lactate, 1.0 g yeast extract, 0.004 g FeCl₃·6H₂O, 0.5 g sodium citrate) in 1 l of double distilled water, which was then adjusted to pH 7.2 with 1 M NaOH and sterilized at 121°C for 20 min (MLS-3780 SANYO, Japan). The medium was inoculated with SRB under anaerobic conditions (Anaerobic chamber, BD) by purging with N₂ at a nominal flow rate for 30 min. The medium was then cultivated at 30°C and 200 rpm for 2 days (VISION Scientific Co., Korea), and used for the corrosion tests along with the control.

The number of IOB and/or SRB in 1 ml of cultivated minimal media, i.e. Sphaerotilus-Leptothrix and Postgate C, was counted under different experimental conditions during the corrosion test. 10–15 ml of each media, which had been sterilized at 121°C for 20 min, was placed in a Petri dish (90 × 15 mm, SPL, Korea), where 1 ml of the sample was inoculated at 10³–10⁵ dilution ratios, and incubated at 30°C for 48 h (VISION Scientific Co., Korea). Subsequently, 30–300 colonies cultivated in each medium were counted as cfu (colony forming unit) ml⁻¹ (ASTM 1998).

**RESULTS AND DISCUSSION**

**Analysis of metal coupons and groundwater samples**

Table 1 describes chemical characteristics of four different metal coupons used in the corrosion tests. An STS 304 coupon contains Cr and Ni and an iron coupon has a dominant amount of Fe. A galvanized steel coupon composes of more Zn than Fe while a zinc steel coupon consists of both Fe and Zn in similar amounts. Table 2 lists the analytical results for the samples from the four pumping wells; B5, B12, B15 and B16. The groundwater samples of B5 and B12 were both neutral pH but the B15 and B16 samples were alkaline, showing pH 10.3 and 8.8, respectively. From ORP, B5 and B12 were potentially under reducing conditions, which is consistent with the low level of DO observed, whereas B15 and B16 were under oxidizing conditions. Although B5 and B12 were in reductive states (negative ORP), the total iron concentration was unexpectedly high at 193.2 and 229.6 mg l⁻¹, respectively. This is possibly due to iron elution or destruction due to pitting corrosion by SRB compared with B15 and B16, which had lower iron concentrations and were in the oxidative state (positive ORP). Although the total iron concentration was different in the four wells, the Fe²⁺ concentration was consistently related to the ORP, where the ORP became more negative with increasing Fe²⁺ concentration. This suggests that the tendency to form iron oxides and their precipitates in the wells could not be determined simply from the ORP and DO concentration. Regardless of the sample location, most wells showed a trace of total organic carbon (TOC), with the highest level of 1.2 mg l⁻¹, indicating the presence
of a rare number of heterotrophic bacterial populations. Microbes present in the samples, as characterized through microbial diversity analysis, were observed under extreme malnutrition conditions or were classified as autotrophic bacteria. As most of the sampling points were located close to the transitional zone of a coastal aquifer, they could be affected by tidal fluctuations, inevitably causing seawater intrusion. Therefore, the Cl⁻ concentration in the wells can vary widely, ranging from 112 to 22,815 mg l⁻¹.

### Table 1 | Chemical characteristics of the metal coupons used in the corrosion tests

<table>
<thead>
<tr>
<th>Material</th>
<th>Stainless steel (STS304)</th>
<th>Iron</th>
<th>Galvanized steel</th>
<th>Zinc steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (mm)</td>
<td>0.35</td>
<td>0.95</td>
<td>0.45</td>
<td>1.15</td>
</tr>
<tr>
<td>Composition (%)</td>
<td>Total 100.00</td>
<td>Total 97.50</td>
<td>Total 92.80</td>
<td>Total 100.00</td>
</tr>
<tr>
<td></td>
<td>Fe 70.80</td>
<td>Fe 97.50</td>
<td>Fe 92.80</td>
<td>Fe 43.60</td>
</tr>
<tr>
<td></td>
<td>Cr 8.50</td>
<td>Mn 1.20</td>
<td>Cl 0.13</td>
<td>Zn 32.50</td>
</tr>
<tr>
<td></td>
<td>Ni 18.70</td>
<td>Si 0.29</td>
<td>S 0.06</td>
<td>P 4.85</td>
</tr>
<tr>
<td></td>
<td>Cu 8.50</td>
<td>Cu 0.15</td>
<td>S 0.05</td>
<td>S 0.18</td>
</tr>
<tr>
<td></td>
<td>Mn 8.50</td>
<td>Co 0.15</td>
<td>Al 0.00</td>
<td>O 0.17</td>
</tr>
<tr>
<td></td>
<td>Si 1.20</td>
<td>Mo 0.09</td>
<td>Mn 0.02</td>
<td>Hg 0.09</td>
</tr>
<tr>
<td></td>
<td>Cr 0.29</td>
<td>V 0.08</td>
<td>Fe 0.01</td>
<td>Fe 0.07</td>
</tr>
<tr>
<td></td>
<td>Mn 0.13</td>
<td>P 0.03</td>
<td>Others 0.01</td>
<td>Ca 0.07</td>
</tr>
<tr>
<td></td>
<td>Si 0.07</td>
<td>Ti 0.01</td>
<td>Others 0.02</td>
<td>Mn 0.07</td>
</tr>
<tr>
<td></td>
<td>Al 0.03</td>
<td>Others</td>
<td>Others 0.02</td>
<td>K 0.05</td>
</tr>
</tbody>
</table>

### Table 2 | Water quality parameters and their concentrations in groundwater samples taken from four bores (B5, B12, B15 and B16) located at the pumping stations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>B5</th>
<th>B12</th>
<th>B15</th>
<th>B16</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.3</td>
<td>6.2</td>
<td>10.3</td>
<td>8.8</td>
</tr>
<tr>
<td>DO (mg l⁻¹)</td>
<td>0.7</td>
<td>1.4</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>-93</td>
<td>-60</td>
<td>8.2</td>
<td>35</td>
</tr>
<tr>
<td>EC (mS cm⁻¹)</td>
<td>18.2</td>
<td>42.2</td>
<td>0.06</td>
<td>34.0</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>43.6</td>
<td>10.6</td>
<td>12.7</td>
<td>33.6</td>
</tr>
<tr>
<td>TOC (mg l⁻¹)</td>
<td>1.17</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Cl⁻ (mg l⁻¹)</td>
<td>2.4 x 10³</td>
<td>2.1 x 10⁴</td>
<td>1.1 x 10³</td>
<td>1.6 x 10⁴</td>
</tr>
<tr>
<td>NO₃⁻ (mg l⁻¹)</td>
<td>132.0</td>
<td>N.D.</td>
<td>887.5</td>
<td>329.3</td>
</tr>
<tr>
<td>SO₄²⁻ (mg l⁻¹)</td>
<td>2.6 x 10²</td>
<td>1.1 x 10³</td>
<td>3.4 x 10³</td>
<td>6.5 x 10²</td>
</tr>
<tr>
<td>Mn²⁺ (mg l⁻¹)</td>
<td>10.3</td>
<td>16.8</td>
<td>N.D.</td>
<td>0.6</td>
</tr>
<tr>
<td>T-Fe (mg l⁻¹)</td>
<td>193.2</td>
<td>229.6</td>
<td>9.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Fe²⁺ (mg l⁻¹)</td>
<td>145.9</td>
<td>65.3</td>
<td>1.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*n.D. – Not detected.*

### Microbial diversity analysis

The microbial community was screened by 16S rDNA analysis, which can determine major types of microbes causing corrosive problems in the pump and pipeline materials in the well locations. Table 3 lists the microbial species found in the analysis, where microbes were observed from B5, B12 and B16. IOB and SRB were never obtained in B15 despite performing a specific primer investigation.

Table 3 summarizes the species physiologically matching more than 98% of the 16S rDNA sequences in the NCBI Database. For that purpose, a total of 96 clones...

### Table 3 | Frequency of microorganisms observed throughout the four monitoring wells of coastal groundwater (B5, B12 and B16) at the pumping stations (16S rRNA gene similarity to the NCBI gene bank database ≥98%)

<table>
<thead>
<tr>
<th>GenBank accession number</th>
<th>Microorganism</th>
<th>B5</th>
<th>B12</th>
<th>B16</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJ012461</td>
<td><em>Sphingomonas echinoides</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AF477498</td>
<td><em>Lactobacillus fermentum</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AM157417</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AY725424</td>
<td><em>Desulfovibrio aespoeensis</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>DQ908951</td>
<td><em>Ralstonia picketii</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AF491333</td>
<td><em>Fusibacter sp. SA1</em></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AJ306752</td>
<td>Uncultured bacterium</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AY570634</td>
<td>Uncultured bacterium</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

*Table 3 summarizes the species physiologically matching more than 98% of the 16S rDNA sequences in the NCBI Database. For that purpose, a total of 96 clones...*
were analyzed. Of these, uncultured bacteria comprised more than 70% of the total, whereas *Sphingomonas echinoides*, which is composed of 20 clones, was dominantly present, except in the uncultured samples. Six *Desulfovibrio aespoeensis* clones were identified, whereas only one each of *Fusibacter* sp. SA1, *Ralstonia pickettii* and *Lactobacillus fermentum* were observed.

*Sphingomonas echinoides* can metabolize a range of organic substrates under a variety of environmental conditions, such that it can be grown in fresh or saline water as well as in a nutrient-deficient system (Lim 2005). *Desulfovibrio aespoeensis* is well reported as an SRB that utilizes lactate, formate and hydrogen as electron donors. On the other hand, it can also metabolize electron acceptors reducing sulfate to H2S in saline water environments (Microbewiki 2007). *Ralstonia pickettii* is an autotrophic bacterial species but is active in the presence of several mM of heavy metals under anaerobic conditions (Lim 2005).

*Desulfovibrio aespoeensis* was chosen as the SRB for the bio-metal corrosion investigation. On the other hand, no IOB were observed by 16S rDNA sequencing, possibly due to the very low DO concentrations that reduced the aerobic microbial population of concern. For that reason, a specific type of primer was used to determine the presence of *Leptothrix mobilis* in the wells through PCR amplification of the genomic DNA product, which is a well known dominant species and an IOB (Emerson & Moyer 1997; Ojumu et al. 2006; Xu et al. 2007). This species was eventually observed in samples from wells B15 and B16, where relatively higher DO concentrations were observed.

The corrosion effect of SRB and IOB on metal coupon

Under 0.01 M NaCl saline conditions, the corrosive effects of microbes on iron coupons were examined in a single culture system. Initially, the populations of IOB and SRB had a similar number ($\sim 2.3 \times 10^5$ cells ml$^{-1}$). The IOB and SRB were incubated under aerobic and anaerobic conditions by air- and N$_2$-purging for 30 min before the test, respectively. On the ninth day after the test had begun, the number of SRB was reduced to one fifth ($4.2 \times 10^4$ cells ml$^{-1}$), whereas that of the IOB was increased by a factor of 3.5 ($8.2 \times 10^5$ cells ml$^{-1}$).

With the mixed culture system, after the 23rd day, the number of SRB in the presence of iron-oxidizing bacteria, which had been incubated in a single reactor, was $4.8 \times 10^6$ cells ml$^{-1}$. This is 66 times higher than that of the sole SRB culture (showing $7.3 \times 10^4$ cells ml$^{-1}$), and was attributed to the prompt increase in the aerobic IOB. On the other hand, with increasing time, the available DO became depleted to as low as 0.1 mg l$^{-1}$, almost reaching anaerobic conditions.

For the SRB cultured alone, the coupon turned a dark black color after the 14th day. The coupon in the mixed culture was also colored dark black after the 22nd day of incubation (Figure 1). Such a dark colored coupon was reported to form when an SRB, i.e., *Desulfovibrio* sp. produces FeS. This will be further accelerated as SRB synthesis increases over an extended period under anaerobic conditions (Kim et al. 2007). In comparison, the coupon with IOB was colored brown red, which might be associated with iron oxidation under aerobic conditions. This was confirmed by XRD analysis, which identified the presence of magnetite (Fe$_3$O$_4$).

Figure 2(a) shows the iron coupons exposed to four different media amended with IOB, SRB or both bacteria.

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**Figure 1** | Photos of the iron coupons after exposure. (a) Iron coupon with SRB injection into the reactor, (b) iron coupon with IOB injection into the reactor.
Regardless of the type of media, the total iron concentration was increasingly leached during the initial experimental stage. However, after the seventh day, the leaching rate for the control was reduced considerably, whereas those of the other coupons remained relatively constant.

Table 4 lists the total and dissolved iron concentrations observed from the corrosion tests in the four different media at the 28th day of incubation. The amended IOB showed the greatest extent of iron leaching into the aqueous medium, 10.2 mg L\(^{-1}\). Ironically, the highest amount of iron precipitates was retrieved from the amended SRB, showing 7.0 mg L\(^{-1}\), but not from the amended IOB. In other words, iron precipitates might be produced more actively due to the formation of FeS being evolved readily during the production of Fe\(_3\)O\(_4\).

Corrosion effects of the microbes on galvanized steel were observed in the 0.01 M NaCl saline environment. Along with the control, the media was amended with iron-oxidizing, sulfur-reducing or both bacteria. Regardless of the type of media, the initial microbial populations were similar, \(3.0 \times 10^5\) cells ml\(^{-1}\). After the seventh day of incubation, the microbial population of the iron-oxidizing bacteria was \(6.2 \times 10^6\) cells ml\(^{-1}\), which is three and thirteen times higher than those of the SRB (\(4.7 \times 10^5\) cells ml\(^{-1}\)) and mixed culture (\(2.3 \times 10^6\) cells ml\(^{-1}\)), respectively. In general,
the microbial population was not related directly to the corrosion rate, which was associated with the oxidation of H₂S induced by the SRB (Beech et al. 1994; Sungur et al. 2007). The relatively lower number of SRB in this experiment might be due to Desulfovibrio desulfuricans being inhibited by zinc, which had a concentration >15 mg l⁻¹ (Poulson et al. 1997). Approximately 40 and 50 mg l⁻¹ of zinc was observed from the SRB and mixed culture, respectively. XRF showed that the zinc concentration in the galvanized steel was originally ~92.8%, which was increased up to three to four times compared with that when the experiment was started, as shown in Figure 2(b). After the seventh day of incubation, the zinc concentration in the control was 5.7 mg l⁻¹, but was reduced to approximately 5.0 mg l⁻¹ at the 28th day. In contrast, those of the other three had increased to approximately 20.0 mg l⁻¹. This suggests that the microbial activity can contribute to the oxidation of the zinc coupon.

The highest concentration of zinc was observed from the media amended with iron-oxidizing bacteria, at 28.8 mg l⁻¹, as listed in Table 4, but the lowest dissolved zinc concentration was 3.4 mg l⁻¹. This indicates that as soon as the zinc is oxidized by the IOB, it might be converted to the fixed forms of precipitates rather than being dissolved.

For the media in a 0.01 M NaCl saline environment, the corrosion effects of the microbes on stainless steel (STS 304) were observed for the control, as well as those amended with IOB, SRB or both bacteria. Regardless of the culture type, similar numbers of microbes were observed initially, approximately 2.3 × 10⁷ cells ml⁻¹. After the ninth day, the number of IOB was ten times higher than that of SRB. This suggests that IOB can grow more competitively than the SRB because many more electron donors were available for the IOB. For the mixed culture, the microbial population, which consisted of IOB or SRB, was ten to twenty times higher than that of the sole cultures. This means that at the start of the test, iron-oxidizing bacteria were initially metabolized actively due to the abundant availability of iron donors. With time, the DO was depleted, which inhibited the aerobic activity, but provided better optimal conditions for the SRB metabolism (Scotto et al. 1985).

The total and dissolved iron concentrations in the solution varied according to the culturing conditions (Figure 2c). The total iron concentration for the control on the 21st day was 0.5 mg l⁻¹, which was far lower than those amended with microbes (i.e., the mixed, 8.9 mg l⁻¹, IOB, 4.5 mg l⁻¹, SRB, 1.2 mg l⁻¹).

After terminating the experiments, all iron species in the sample, such as total, dissolved and precipitated iron, were analyzed (Table 4). The highest total iron concentration was observed in the mixed culture (1.8 mg l⁻¹). Similar precipitated iron levels were observed in both the SRB and control experiments (~0.3 mg l⁻¹). Therefore, the SRB could not significantly affect the corrosion of stainless steel. The highest concentration of iron precipitates was acquired from the iron oxidizing medium (1.54 mg l⁻¹), which was higher than that of the mixed culture (1.47 mg l⁻¹). This suggests that the corrosion of stainless steel could be dominated by the IOB, whereas the metabolism of the IOB in the mixed culture may be hindered by the more competitive activity of the SRB when oxygen has been depleted with increasing incubation time. Xu et al. (2007) reported that 243 mg l⁻¹ of Cl⁻ can more adversely affect the corrosion of 316 L stainless steel in the presence of either SRB or IOB than that of the control. Furthermore, more precipitates were produced in the mixed culture when both IOB and SRB were present.

The corrosion effects of the microbes on zinc steel were investigated in a 0.01 M NaCl saline environment. Initially, the three different cultures (e.g., IOB, SRB and mixed) had similar microbial populations (~3.0 × 10⁷ cells ml⁻¹). On the seventh day, the population of IOB outnumbered those of either the SRB or mixed culture by approximately ten times. This suggests that the iron donor supplied from the oxidation of iron is more likely to help stimulate the growth of IOB than that of SRB or IOB in the mixed culture. In the mixed culture, the environment for the IOB might have been inferior due to a temporal change in the respiration conditions, as mentioned earlier.

XRF revealed the coupon to be composed of 43.6 and 32.5% iron and zinc, respectively, showing an 11% greater concentration of iron than zinc. On the other hand, 7 days after the test began, the concentration of iron and zinc released from the IOB was 1.2 and 18.5 mg l⁻¹, respectively. This indicates that the corrosion rate of zinc was much faster than that of iron. As shown in Figure 2(d), during the first 7 days of the experiment amended with the IOB,
zinc was the species most likely to be leached from the coupon. Finally, on the 28th day, the highest iron concentration was leached from the mixed culture (2.1 mg l\(^{-1}\)). In contrast, 19.6 mg l\(^{-1}\) of zinc was observed from the SRB. On day 26th, the test was terminated and the total and dissolved zinc concentrations in the sample were determined, as shown in Table 4. The leaching of zinc was highest (i.e., 19.6 mg l\(^{-1}\)) for the SRB, which was attributed to the highest amount of precipitates formed (14.3 mg l\(^{-1}\)). This means that the corrosion of zinc steel is most likely to have been affected by the presence of SRB.

Overall, regardless of the coupon type, the extent of corrosion decreased with increasing reaction time. Corrosion appeared to be temporally terminated in the closed system compared with that in an open system, which would consistently extend to further corrosion (Sungur et al. 2007).

Deactivation of SRB and IOB in the medium with the metal coupon

The IOB and SRB were disinfected by either NaOCl or UV illumination during incubation of the iron coupon submerged in the media, as shown in Figures 3(a) and (b). These figures show the rate of microbial die-off (i.e., IOB and SRB) when exposed to either a nominal concentration of NaOCl or UV irradiation. The iron concentration in the solution was also monitored as disinfection progressed.

The inactivation efficiency was assessed by comparing the microbial population in the sample with that in the blank. When the population in the former outnumbered that in the latter, it was considered as a negative value. A positive value was denoted when the microbes had been deactivated more effectively. Under UV irradiation, 98% of
the SRB died off after 3 days of contact, but the IOB showed an erratic pattern of disinfection with exposure time despite the greatest efficiency of 95% being observed after two days illumination. Therefore, no apparent time dependent pattern of deactivation was observed in the IOB under UV illumination.

In Figure 3, a greater decline in the total iron concentration than that of the control was marked as a positive value, and a negative value in the opposite case. As shown in Figures 3(a) and (b), the total iron concentrations released into solution were reduced to 143 and 562% when SRB were exposed to UV illumination for three days and 200 ppmv NaOCl, respectively. This suggests that NaOCl can more effectively inhibit the growth of SRB than UV irradiation. On the other hand, the levels of iron released were increased markedly to 326 and 728% when IOB were exposed to UV illumination for one day and 200 ppmv NaOCl, respectively. Consequently, the SRB, *Desulfovibrio* sp., can be deactivated by an injection of NaOCl or with UV irradiation. Nevertheless, the use of these methods did not effectively disinfect the IOB but instead caused an increase in iron corrosion.

Figures 3(c) and (d) show the inactivity test results for the IOB and SRB with a nominal concentration of NaOCl or UV illumination when the stainless steel coupon was dipped into the media. As shown in Figure 3(c), under UV irradiation, the number of SRB was increased by 65% after two days of illumination but was decreased by 103% after three days exposure. In comparison, the number of IOB was reduced considerably by 960% after three days irradiation. This suggests that the IOB can be disinfected more effectively by UV exposure than SRB. Figure 3(d) shows that the addition of 2 ppmv NaOCl could not effectively disinfect the SRB. The population of SRB was increased by 50%. In contrast, the addition of NaOCl was more effective in disinfecting IOB, which were reduced by 9 and 616% with 2 and 200 ppmv NaOCl, respectively.

The total iron concentration was increased by 103 and 110% despite the SRB being disinfected by one day of UV illumination or the addition of 2 ppmv NaOCl, respectively (Figures 3(c) and (d)). On the other hand, it was reduced when the illumination duration was expanded to two and three days or when the NaOCl concentration was increased to 20 and 200 ppmv. Therefore, the disinfection of the SRB can decrease the extent of iron oxidation by their effective deactivation (*Desulfovibrio* sp.) when a STS 304 coupon is dipped into the media.

As shown in Figures 3(c) and (d), the total iron concentration was reduced by 594 and 87% when the IOB was disinfected for one and three days with UV illumination, respectively. In addition, the dosage of 200 ppmv NaOCl reduced the total iron concentration by 124%. Nevertheless, the addition of 2 ppmv NaOCl increased the total iron concentration to 135 μg l⁻¹, which was 531% higher than the control. This can be explained by the difference in the number of microbes affected by the addition of 2 ppmv NaOCl, which decreased the microbial population by 9%, compared with the addition of 200 ppmv NaOCl, which reduced the total iron concentration by 616%.

**CONCLUSION**

The bio-corrosion of four different types of metal coupons in saline media were investigated using the IOB, *Leptothrix* sp., and SRB, *Desulfovibrio* sp. The corrosion characteristics were addressed with regard to the microbial population, aqueous phase iron concentration, precipitate formation, and microbial inactivation. Overall, iron and zinc steels were much more vulnerable to attack by biologically mediated corrosion than STS 304 and galvanized steels. Moreover, zinc steel showed the highest level of corrosion. In response, higher amounts of corrosion precipitates were produced from the oxidation of iron and zinc steel.

In a single culture, iron was corroded more severely by the IOB, but a larger amount of iron precipitates was produced with the SRB. Galvanized steel was found to be more vulnerable to corrosion by IOB than SRB. The SRB also brought about a larger amount of precipitates than the IOB. Stainless steel (STS 304) was also corroded more severely by IOB than SRB.

In the mixed culture, the populations of IOB and SRB were outnumbered by the potential synergetic effects compared with a single culture. This can increase the degrees of corrosion by factors of two and seven compared with that of the single IOB and SRB cultures, respectively. Zinc steel contained 43.6 and 32.5% of iron and zinc, respectively. Nevertheless, zinc was more highly oxidized than
iron (by a factor of 15), indicating that zinc would be eroded more readily. This can also be explained by the weaker redox potential of zinc (−0.76 V) than iron (−0.44 V), meaning that iron is more resistant to corrosion than zinc.

The addition of NaOCl or UV irradiation reduced the level of microbial corrosion efficiently in the presence of iron and stainless steel coupons. On the other hand, the overdosing of NaOCl might increase the level of corrosion without properly controlling the number of microbes. In particular, for the IOB, the insufficient addition of NaOCl would result in persistently growing microbes, leading to an increase in the tendency for corrosion (e.g. an iron-coupon with 200 ppmv NaOCl and a stainless steel coupon with 2 ppmv NaOCl). This means that the addition of NaOCl must be dosed carefully and optimally to critically control the microbial activity and limit corrosion. In other words, an uncontrolled dosage of NaOCl exceeding an optimal point or scattered UV light due to suspended particles could chemically or biologically exacerbate metal corrosion.

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