Impact and Role of Bacterial Communities on Biocorrosion of Metals Used in the Processing Industry

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ABSTRACT: In the present study, the effects of the corrosive bacterial community and the biofilm on cooling water systems made from mild steel (MS) and brass (BR) were studied under field exposure conditions using electrochemical impedance spectroscopy measurements, scanning electron microscope, and X-ray diffraction methods. Results from 16S rRNA gene sequences showed that the predominant bacteria species detected in the biofilm of MS and BR metals during 360 days of exposure were Bacillus cereus EN14, Achromobacter xylosoxidans EN15, A. xylosoxidans EN16, and B. cereus EN17. The weight loss results revealed that a higher corrosion rate was observed in MS (0.7 ± 0.1 mm/y) compared with that in BR (0.17 ± 0.05 mm/y) at the end of the exposure period. This can be explained by the bacterial communities enhancing the corrosion rates by creating a local corrosive environment. Scanning electron microscope images revealed the adsorption of biofilm on the MS and BR surfaces following 180 days of exposure. From the electrochemical impedance study, a higher charge transfer resistance ($R_{ct}$) was obtained for BR (119.6 Ω cm$^2$) when compared with that of MS (43.4 Ω cm$^2$). This study explains the role of bacterial communities and their mechanisms in the corrosion of MS and BR in cooling water systems.

INTRODUCTION

Corrosion is broadly defined as the deterioration of a metal surface due to the interaction between metals and their environment. Microbially influenced corrosion (MIC) is an electrochemical reaction that enhances the corrosion process due to microbial metabolites (sulfide, organic acids, inorganic acids, and ammonia). Bacterial metabolites change the environmental conditions (such as salinity, acidity, and oxygen concentration) around metal surfaces, which lead to the formation of corrosion on the materials. MIC is often associated with iron-oxidizing bacteria, iron-reducing bacteria, sulfate-reducing bacteria (SRB), acid-producing bacteria, and fungi. Cooling water systems (CWSs) are used to remove and dissipate wasted heat to the surrounding environment. The industrial systems consist of cooling towers, pipeline circulation water, pumps, and heat exchangers. In general, cooling towers remove heat through evaporation, and dissolved constituents are removed in the recirculating water. The recirculation water has a higher content of recycled water than fresh water. This promotes biofilm formation and corrosion. Biocorrosion has significant economic and ecological impacts. Several models have been documented to describe its fundamental mechanisms.

Extracellular polymeric substances (EPSs) play an important role in facilitating microbial cell adhesion to iron surfaces and form biofilms on metal surfaces. EPSs are believed to be polymeric conglomerations (proteins, polysaccharides, and lipids), which lead to the biosorption ability to iron ions. In general, EPSs consist of functional groups that can easily bind to metal surfaces, thereby increasing the corrosion rates due to the biofilm, and result in the enhancement of cathodic reactions. Currently, mild steel (MS) and brass (BR) are used extensively as metals in CWSs due to their excellent insulation properties, easy construction properties, and low cost.

The rate of metal corrosion is impacted by the deposition of gaseous compounds, relative humidity (RH), temperature, moisture, and biological aerosols on the metal surface. The effect of continuous microbial growth will result in reduction of water flow, thereby leading to system failures. Hence, in the current study, we investigated the role of bacterial communities on the corrosion of MS and BR in CWSs under field conditions. The bacterial biofilms formed on MS and BR
were characterized using 16S rRNA analysis. The rate of corrosion was assessed by weight loss (WL) and impedance/polarization methods. SEM and XRD surface analyses were used to characterize the biofilms and corrosion products.

### RESULTS AND DISCUSSION

#### Bacteriological Analysis of Cooling Water System

Preliminary identification of the isolates by biochemical tests reveals that EN14 and EN17 are reported as facultative Gram-positive, aerobic, rod-shaped, catalase-producing, and oxidase-positive bacteria. EN15 and EN16 are reported as Gram-negative, rod-shaped, motile, catalase-producing, and oxidase-positive bacteria. The biochemical characterization results are presented in Table 2. The obtained sequence was aligned and compared with GenBank. The results showed >99% homology with *Bacillus cereus* and *Achromobacter xylosoxidans*. Hence, these organisms have been named *B. cereus* EN14, *A. xylosoxidans* EN15, *A. xylosoxidans* EN16, and *B. cereus* EN17. The sequences were subjected to a BLAST search to repossess the related species by the taxonomy and categorization packing order in accordance with the NCBI tools. Figure 1 shows the cluster-tree analysis of the relationship between isolates and related species. The EN14, EN15, EN16, and EN17 gene sequences were submitted to GenBank, and accession numbers of MF803659, MF803660, MF803661 and MF803662, respectively, were obtained.

#### Biocorrosion and Surface Analysis

The WL and corresponding corrosion rates of MS and BR are presented in Table 3. In MS, an average WL of 2.7 ± 0.6 g and an average corrosion rate (CR) of 0.7 ± 0.01 mm/year were obtained following an exposure period of 360 days. During the initial 90 day exposure period, the CR of MS was determined to be 0.729 ± 0.02 mm/year, and the rate steadily increased to 1.054 ± 0.5 mm/year after an additional 90 days of exposure following the initial 90 days of exposure. However, during the next 180 days, the CR decreased to 0.7 ± 0.10 mm/year due to its re-passivation of iron oxide and it might lead to the decreased biofilm activity, thus reducing corrosion. The atmospheric environmental CR decreased with long exposure time. This result is supported by earlier reports.

The results for BR were similar to those for MS. Values of WL and corresponding CR are presented in Table 3. The average WL and CR values were determined to be 0.7 ± 0.1 g and 0.17 ± 0.05 mm/year, respectively, at the end of the exposure period. Following the initial 90 day exposure period, the WL of BR was determined to be 0.141 ± 0.01 g and constantly increased thereafter to 0.631 ± 0.20 g after a total of 270 days. The WL was higher for MS when compared with BR over the exposure period. The initial CR was higher and could be attributable to the presence of biofilms and sessile cells that are directly in contact with the metal surface, thereby creating a condition that is favorable to corrosion. Conversely, it is not inconceivable that, after the initial exposure period, a protective layer had formed on the metal surface, which led to limited contact of the metal with the microbes and a reduction in the CR.

*A. xylosoxidans* and *B. cereus* are catalase-producing bacteria. Their related biochemical characterizations are presented in Table 2. A bacterial catalase enzyme was used to neutralize the oxygen and the oxidation of metal ions. This process is termed catalase-mediated corrosion. The chloride concentration of

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**Table 1. Physiochemical Parameter of Water in Cooling Tower**

| days | pH  | alkalinity (mg/L) | hardness (mg/L) | chloride ion (mg/L) | total sulfate (mg/L) | total nitrate (mg/L) |
|------|-----|-------------------|-----------------|---------------------|----------------------|----------------------|
| 90   | 6.5 | 19                | 32              | 40                  | 12                   | 42                   |
| 180  | 6.0 | 27                | 44              | 64                  | 20                   | 45                   |
| 270  | 5.5 | 17                | 56              | 120                 | 15                   | 45                   |
| 360  | 6.0–7.0 | 10–30           | 61              | 200                 | 10–15                | 44                   |

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**Table 2. Biochemical Characterization of Bacillus-Related Species from Cooling Tower Water**

| characteristics | EN14 | EN15 | EN16 | EN17 |
|-----------------|------|------|------|------|
| Gram stain      | positive | negative | negative | positive |
| shape           | rod   | rod   | rod   | rod   |
| motility        | +     | +     | +     | +     |
| sporulation     | –     | +     | –     | +     |
| growth          | at 20 °C | –   | –     | –     |
|                 | at 30 °C | +     | +     | +     |
|                 | at 40 °C | +     | +     | +     |
| indole test     | –     | –     | –     | –     |
| methyl red test | +     | +     | +     | +     |
| Voges–Proskauer test | +     | –     | +     | +     |
| citrate utilization test | –     | +     | –     | –     |
| oxidase test    | +     | +     | –     | –     |
| catalase test   | +     | +     | +     | +     |
| production of acid from glucose | +     | +     | +     | +     |
| galactose       | +     | –     | –     | –     |
| fructose        | +     | +     | +     | +     |
| sucrose         | –     | +     | –     | –     |
| hydrolysis of starch | +     | +     | +     | +     |
| cellulose       | +     | +     | +     | +     |
| casein          | –     | –     | –     | –     |
| urea            | +     | +     | +     | +     |

\(+\), positive response; \((-\)), negative response.

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**Figure 1. Cluster-tree analysis of the bacterial community in cooling water systems by 16S rRNA gene sequences: (A) *Bacillus* sp. and (B) *Achromobacter* sp.**
the cooling tower water was found to increase during the exposure period. The higher concentration of chloride (200 mg/L) was noticed at the end of the immersion. This observation reveals that chloride ions also contribute to the observed corrosion in both metals.

This bacterial community is able to consume oxygen and produce water molecules (eq 1).

\[
\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O
\]  

(1)

The bacterial biofilm supports the Fenton reaction\(^{32}\) by reducing the metal ions, which leads to the formation of hydroxyl radicals (eq 2). The Fe\(^{3+}\) ions produced from the reaction further react with OH\(^-\) ions to form ferric hydroxide (eq 3) as a corrosion product on metal surfaces.

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + 2OH^-
\]  

(2)

\[
Fe^{2+} + 2HO \rightarrow Fe(OH)_2
\]  

(3)

XRD data from the corrosion product collected from the cooling water exposure of MS and BR metals are presented in Figure 2. Ferrous sulfide (FeS), iron oxychloride (FeOCl), iron hydroxide (FeOOH), and iron oxide (Fe\(_2\)O\(_3\)) were observed (Figure 2Aa). Copper oxide (CuO\(_2\)) and Cu(OH)\(_2\) (Figure 2Ba) were observed in the initial 90 day exposure period. Upon increasing the exposure period (180 days), higher intensities of Fe\(_2\)O\(_3\), FeOCl, and Cu\(_2\)O were observed (Figure 2Ab, Bb), which indicates the higher rate of corrosion on the metal surface. On the other hand, a prolonged exposure period led to a decrease in the intensity of the peaks due to the re-passivation of the surface film. This phenomenon occurred up until the end of the exposure period (360 days).

The SEM of MS and BR at various exposure times (Figure 3) showed the development of a bacterial biofilm and its increase over time. Thicker biofilm formation was observed from SEM results for both MS and BR after 180 days (Figure 3c). The occurrence of MIC is concurrent with the production of EPSs and cellular adhesion during biofilm formation on the metal surface. These processes lead to a significant alteration of the metal interface, which acts as a barrier to the swapping of elements between the aqueous phase and metal surface. The higher production of EPSs on the metal surface results in differential aeration and alteration of the pH and redox reactions (potential difference) on the metal surfaces, which ultimately leads to the corrosion process.\(^{33}\)

**Electrochemical Studies.** The impedance curve for the MS and BR metals at different exposure periods is shown in Figure 4; associated data is presented in Table 4. The Nyquist curves for MS and BR exhibited a semicircle at the initial exposure period (90 days). After 90 days, a depressed semicircle was observed and charge transfer resistance \((R_{ct})\) values for MS and BR were lower (461 and 710 \(\Omega\) cm\(^2\), respectively) than values obtained from the initial exposure period (978.5 and 1576 \(\Omega\) cm\(^2\), respectively). This indicated the occurrence of corrosion reactions on the metal surfaces.\(^{34-36}\) Following 270 days of exposure, the \(R_{ct}\) values for MS and BR were found to be higher (966.9 and 1466.5 \(\Omega\) cm\(^2\), respectively) than values obtained after 180 days. This indicates a decrease in the corrosion rate on the metal surface;\(^{37}\) Natesan et al.\(^{30}\) reported that a prolonged exposure period led the development of a thick corrosion product layer on the metal surface, and thus limiting the contact

| metal     | WL (g)     | CR (mm/year) | WL (g)     | CR (mm/year) | WL (g)     | CR (mm/year) | WL (g)     | CR (mm/year) |
|-----------|------------|--------------|------------|--------------|------------|--------------|------------|--------------|
| mild steel| 0.703 ± 0.1| 0.729 ± 0.2  | 2.166 ± 0.7| 1.054 ± 0.5  | 2.037 ± 0.8| 0.704 ± 0.32| 2.7 ± 0.6  | 0.7 ± 0.1    |
| brass     | 0.141 ± 0.01| 0.137 ± 0.02| 0.266 ± 0.03| 0.1295 ± 0.1| 0.631 ± 0.2 | 0.204 ± 0.03| 0.7 ± 0.1  | 0.17 ± 0.05  |

Figure 2. XRD pattern of (A) mild steel (B) brass at different immersion periods: (a) 90, (b) 180, (c) 270, and (d) 360 days.
of corrosive bacterial species with the metal surface, thereby reducing the CR.

Tafel polarization curves and data for MS and BR are presented in Figure 5 and Table 5, respectively. During the initial exposure period, polarization resistances ($R_p$) for MS and BR were approximately 2.4 and 2.4 kΩ cm$^2$, respectively. However, after 90 days, the $R_p$ values (1.3 and 2 kΩ cm$^2$, respectively) were shown to have decreased. In contrast, during the initial 90 day

Figure 3. SEM/EDS analysis of the MS/BR surface coupons after immersion at various incubation periods: (a) 90, (b) 180, (c) 270, and (d) 360 days.
exposure period, corrosion current values ($i_{corr}$) for MS and BR (46 and 14 $\mu$A/cm$^2$, respectively) were less than the values obtained after 90 days (77 and 69 $\mu$A/cm$^2$, respectively). This can be explained by the formation of a corrosion film by bacterial species.38,39,45 Remarkably, following 270 days of exposure, the $i_{corr}$ values for MS and BR (30 and 61 $\mu$A/cm$^2$, respectively) and $R_p$ values (4 and 3 k$\Omega$ cm$^2$, respectively) were less than values obtained after 180 days. Hence, it can be concluded that the corrosion rate decreased after longer times of exposure. These results are further substantiated by other studies.28,40 Thus, the EIS results also supported the WL studies and confirmed the higher corrosion rates that were found to occur during the initial exposure period compared with the end of the exposure period (360 days). MS was found to have a higher corrosion rate when compared to BR.

### Table 4. Electrochemical Impedance Parameters for Mild Steel 1010 and Brass Coupons at Different Exposure Times at Cooling Tower Water

| Duration   | Mild Steel | Brass |
|------------|------------|-------|
| R_s (Ω)    | R_ct (Ω cm$^2$) | R_s (Ω) | R_ct (Ω cm$^2$) |
| 90 days    | 31         | 978.5  | 42      | 1576   |
| 180 days   | 10         | 461    | 14      | 710    |
| 270 days   | 21         | 966.9  | 35      | 1466.5 |
| 360 days   | 2          | 43.4   | 20      | 119.6  |

*$R_s$, solution resistance; $R_{ct}$, charge transfer resistance.

### CONCLUSIONS

The present study investigated the role of a bacterial biofilm on CWSs made of MS and BR. A. xylosoxidans and B. cereus were identified as the corrosive bacteria species on metal surfaces in the CWS that were examined by WL, XRD, SEM, and EIS. XRD confirmed the presence of Fe and Cu oxide as part of the corrosion products. This indicated that the isolated bacteria are able to convert the elements on the MS and BR surfaces. The result was further corroborated by the electrochemical data (EIS and polarization). The results from this study demonstrated that bacteria accelerate the corrosion of MS and BR due to bacterial metabolism. Results from the WL experiment confirmed that BR has a higher resistance of corrosion than MS. This study demonstrated that the bacterial community enhances the corrosion of CWSs made of various engineering materials. This study contributes to an expansion of the current knowledge pertaining to the role of bacteria and the mechanisms of corrosion that are essential to the selection of biocides or corrosion inhibitors that can be used to control corrosion in processing industries.

### MATERIALS AND METHOD

#### Background Information of the Cooling Tower Water.

The processing facility (cooling tower) used in this study is located at a chemical processing site in Sipcot, Ranipet, Vellore district, Tamilnadu, India (latitude, 12.953671° and longitude, 79.313132°). The tower has a dimension of 3.4 m x 2.8 m x 1.0
Table 5. Polarization Parameters for Mild Steel 1010 and Brass Coupons at Different Exposure Times at Cooling Tower Water\textsuperscript{a}

| duration  | \(E_{corr}\) (\(\mu A/cm^2\)) | \(i_{corr}\) (\(\mu A/cm^2\)) | \(\beta_p\) (mV/dec) | \(\beta_c\) (mV/dec) | \(R_p\) (k\(\Omega\) cm\(^2\)) | \(E_{corr}\) (\(\mu A/cm^2\)) | \(i_{corr}\) (\(\mu A/cm^2\)) | \(\beta_p\) (mV/dec) | \(\beta_c\) (mV/dec) | \(R_p\) (k\(\Omega\) cm\(^2\)) |
|-----------|-------------------------------|-------------------------------|--------------------|--------------------|-----------------|-------------------------------|-------------------------------|--------------------|--------------------|-----------------|
| 90 days   | 523                           | 46                            | 582                | 470                | 2.4             | 69                            | 14                            | 100                | 344                | 2.4             |
| 180 days  | 367                           | 77                            | 523                | 462                | 1.3             | 138                           | 69                            | 921                | 512                | 2               |
| 270 days  | 142                           | 30                            | 674                | 475                | 4               | 105                           | 61                            | 529                | 497                | 3               |
| 360 days  | 123                           | 39                            | 791                | 482                | 3.3             | 122                           | 72                            | 428                | 465                | 1.3             |

\(\textit{a}\)\(E_{corr}\), corrosion potential; \(i_{corr}\), corrosion current; \(\beta_p\), anodic slope; \(\beta_c\), cathodic slope; and \(R_p\), polarization resistance.

m (water depth) and a capacity of 1000 m\(^3\)/h. The temperature was maintained at \(\sim 31.2 \, ^\circ\text{C}\). Physicochemical characteristics of the water are presented in Table 1.

**Preparation of Metal Samples.** Coupons of MS 1010 (0.16% carbon, 0.37% silicon, 1.24% manganese, 0.027% phosphorus, 0.026% sulfur, 0.19% copper, 0.007% nitrogen, 0.02% aluminum, and 97.96% iron) and BR coupons (16% zinc, 0.05% iron, 0.05% lead, and 60.66% copper) were exposed to the cooling tower water system to examine the susceptibility of the metal to biofilm formation. Each metal coupon was sequentially ground with a series of grit silicon carbide papers (grades 180, 250, 500, 800, 1200, and 1500) to produce a smooth metal surface and ultimately polished to a mirror surface using micron alumina (0.3) powder. The refined coupons were rinsed with distilled water, and further surface sterilization was carried out by immersing in 70% ethanol solution for 1 min. After sterilization, the coupons were stored in a desiccator until they were ready for further use. Two different sizes of metal coupons were used in the present investigation: a round coupon with a diameter of 10 mm for EIS analysis and a 7.5 cm \(\times\) 1 cm \(\times\) 0.1 cm coupon for the weight loss experiment.\textsuperscript{41}

Polished MS 1010 and BR metal coupons were exposed to the cooling tower water system under field conditions. After different time intervals of 90, 180, 270, and 360 days, both types of coupons were removed from the cooling tower water and subjected to 16S RNA, WL, EIS, XRD, and SEM analyses.

**Bacterial Community Analysis.** Biofilm samples were collected from the surface of immersed metal coupons (MS and BR) and analyzed for identification of aerobic bacteria. The biofilm samples were serially diluted (10-fold) and plated using a pour plate technique on the following sterile selective media: American Petroleum Institute (API) medium, iron-oxidizing medium (IOM), and manganese bacterial agar (MA).\textsuperscript{2} The total viable counts were enumerated on each sterile selective agar plates, and bacterial populations were articulated as colony-forming units (CFU/mL) after an incubation period of 24–48 hours at 37 \(^\circ\text{C}\). Dissimilar morphology bacterial colonies were selected randomly and purified by the streak plate method on specific sterile agar plates. The resulting bacterial isolates were identified according to (1) Gram staining, (2) motility test, (3) indole production, (4) methyl red test, (5) citrate utilization test, (6) Voges–Proskauer test, (7) carbohydrate fermentation test, (8) catalase test, (9) oxidase test, (10) gelatin content, (11) starch content, and (12) lipid hydrolysis test.\textsuperscript{32}

**Molecular Identification of Aerobic Bacteria and Phylogenetic Analysis.** Bacterial genomic DNA extraction was adopted from the work of Weisberg et al.\textsuperscript{53} Bacterial genomic DNA was subjected to amplification by polymerase chain reaction (PCR) using universal primers, 16S RNA forward primer 5′-AGAGTTTGATCCTGCTCAG-3′ and reverse primer 5′-ACGGCTACCTTGTGCTAC-3′.\textsuperscript{44,45} PCR was performed with 50 \(\mu\)L of reaction mixture containing 1.5 mM MgCl\(_2\), 2 \(\mu\)L of template DNA, forward and reverse primers (0.5 \(\mu\)M dNTP at a concentration of 50 \(\mu\)M, 1\(\mu\)L of TaqDNA polymerase, and 5\(\mu\)L of buffer as recommended by the manufacturer, MBI Fermentas), the reaction was adopted from ref 2. PCR products were purified using a Montage PCR Cleanup kit (Millipore) for DNA sequencing and using a Big Dye terminator cycle sequencing kit (Applied BioSystems, USA), and the obtained bacterial 16S RNA sequences were determined using an automated DNA sequencing system (model 3730XL, Applied BioSystems, USA).

The obtained sequences were examined by a BLAST search (version 2.2.20\textsuperscript{46}), and the taxonomic hierarchy of the sequences was identified using the Ribosomal Database Project II Release 10 (http://rdp.cme.msu.edu). A taxonomically related sequence was acquired from the National Centre for Biotechnology Information (NCBI) taxonomy database and Ribosomal Database Project-II (Release 10). Phylogenetic association and sequence resemblance analysis were performed on frequent 16S RNA gene regions, and misaligned sequences were treated using the MEGA software program (version 4.1).\textsuperscript{27} Phylogenetic trees were constructed by a neighbor-joining method, and Mat GAT (version 2.01) was used to analyze the similitude percentages between sequences.

**Biocorrosion and Surface Analysis.** For the weight loss experiment, the metal coupons were pickled with Clark solution followed by drying with an air drier. Triplicate experiments were conducted for each system. The final weights of each coupon were taken in every 90 day time interval, and the average corrosion rates were calculated following the NACE standard protocol (NACE 2005). At the end of the corrosion experiment, the corrosion products were carefully collected and subjected to surface analysis (XRD and SEM). The corrosion products were crushed into fine powder and used for XRD analysis. A system controlled XRD (Bruker-8030) between 29 of 10 to 85\(^\circ\) was used.\textsuperscript{37} SEM analyses were carried out in a JEOL JSM6000 to assess the bacterial grouping. The biofilm was fixed on the metal surface using 3% glutaraldehyde in a phosphate buffer solution, left overnight, and washed with different amounts of ethanol (25, 50, 75, and 95%). The metal sample was dried and subjected to SEM; this analysis was conducted at time intervals of 90 days during the incubation period.\textsuperscript{38}

**Electrochemical Studies.** Corrosion characterization of MS and BR metal coupons was carried out by EIS. Impedance and polarization were conducted in a three-electrode electrochemical cell (CH Instrument Inc., USA model CHI 608E), with the MS and BR metal coupon as the working electrode, Ag/AgCl as the reference electrode, and a platinum wire as the counter electrode. EIS was performed at an open circuit potential (OCP) using a 10 mV amplitude sinusoidal signal over frequencies ranging from 5 mHz to 100 kHz. Polarization was determined at a scan rate of 0.5 mV/s. Anodic and cathodic curves were obtained by scanning from the OCP toward an OCP of +200 mV anodically and an OCP of −200 mV cathodically.\textsuperscript{39–51}
Notes
The authors declare no competing financial interest.

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