**ORIGINAL ARTICLE**

# Control of corrosive bacterial community by bronopol in industrial water system

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## Abstract

Ten aerobic corrosive bacterial strains were isolated from a cooling tower water system (CWS) which were identified based on the biochemical characterization and 16S rRNA gene sequencing. Out of them, dominant corrosion-causing bacteria, namely, *Bacillus thuringiensis* EN2, *Terribacillus aidingensis* EN3, and *Bacillus oleronius* EN9, were selected for biocorrosion studies on mild steel 1010 (MS) in a CWS. The biocorrosion behaviour of EN2, EN3, and EN9 strains was studied using immersion test (weight loss method), electrochemical analysis, and surface analysis. To address the corrosion problems, an anti-corrosive study using a biocide, bronopol was also demonstrated. Scanning electron microscopy and Fourier-transform infrared spectroscopy analyses of the MS coupons with biofilm developed after exposure to CWS confirmed the accumulation of extracellular polymeric substances and revealed that biofilms was formed as microcolonies, which subsequently cause pitting corrosion. In contrast, the biocide system, no pitting type of corrosion, was observed and weight loss was reduced about $32 \pm 2$ mg over biotic system ($286 \pm 2$ mg). FTIR results confirmed the adsorption of bronopol on the MS metal surface as protective layer (co-ordination of $NH_2$–$Fe^{3+}$) to prevent the biofilm formation and inhibit the corrosive chemical compounds and thus led to reduction of corrosion rate ($10 \pm 1$ mm/year). Overall, the results from WL, EIS, SEM, XRD, and FTIR concluded that bronopol was identified as effective biocide and corrosion inhibitor which controls the both chemical and biocorrosion of MS in CWS.

**Graphical Abstract**
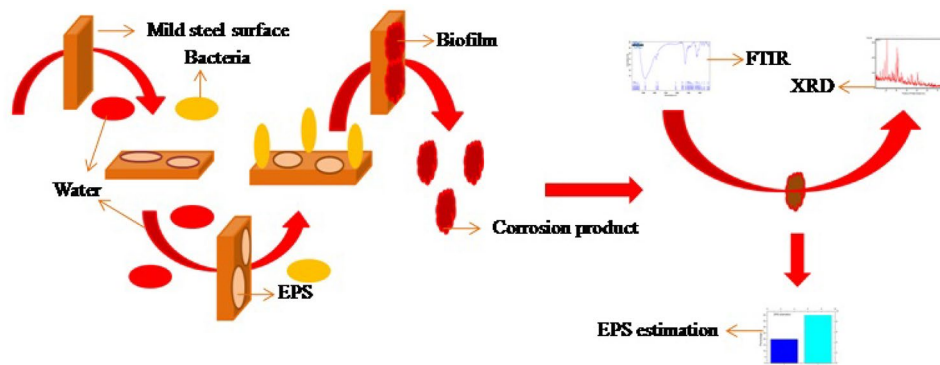
## Introduction

Biofilm development is one of the most common problem in cooling tower water systems. This can lead to excessive damage to expensive equipments/instruments, thus

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increasing the maintenance costs in the industrial sectors. Cooling towers are frequently used to dissipate heat from recirculating water to the atmosphere by means of trickling or spraying the water over a material with high surface area (MacDonald and Brozel 2000; Narenkumar et al. 2017). In a recirculating cooling water system, the temperature is generally maintained between 20 and 40 °C, an environmental condition that favors microbial growth (Wagner and Little 1993; Xu 2012). Microbial influenced corrosion (MIC) refers to the corrosion induced by the metabolic activity of microorganisms on the surfaces of both metals and non-metallic materials. The microorganisms influence corrosion by altering the chemistry between the metal and the bulk fluid at the interface (Narenkumar et al. 2017; Okabe et al. 1994; Palaniappan and Toleti 2015; Booth 1971; Parthipan et al. 2017a, b; Rajasekar et al. 2017). Microbial corrosion is considered to be a leading cause of pipe failures, thus increasing the operating and maintenance costs for recirculating water plants (Flemming 1996).

Most of the microorganisms associated with MIC includes sulphate reducing/oxidizing bacteria (Rosa et al. 2016), iron reducing/oxidizing bacteria (Beech and Gaylarde 1999), polysaccharide producing bacteria, acid producing bacteria, a group of *Bacillus* sp. (Morikawa 2006), and fungi (Fontana 1986). A biofilm is a microbial community of cells embedded in a thick mucilaginous matrix of extracellular polymeric substances (EPS) on the metal surface. It consists of 90% water and remaining 10% of polysaccharides, protein and lipids, etc. (Lewis 2001). EPS are the major structural and physical components of biofilm formation on the metallic and non-metallic surfaces (Jayaraman et al. 1997; Harrah et al. 2004). EPS also influence the biofilm life cycle process by accelerating the redox reactions on the surface of the material (Padmavathi et al. 2015; Frank and Belfort 2003; Walker et al. 2005; Liu et al. 2007; Rajasekar and Ting 2011). This process leads to corrosion reaction on the metal surface. Biocorrosion occurs in various processing sectors, such as water treatment system, distribution, and operating system of cooling water towers (Bott et al. 1983; Challinor 1991). Several chemicals/biocides (citric acid, hydroxy ethylidene diphosphonic acid, acrylate copolymer, and isothiozolone) are used to control MIC in cooling tower materials (Schwermer et al. 2008). As reported by many researchers, an effective control of MIC can be achieved through the application of effective biocides containing nitrate or nitrite component (Wen et al. 2009; Haveman et al. 2004). Nitrite is a relatively in expensive and reported to specifically inhibit the sulphate reducing corrosive bacteria (Greene et al. 2003, 2006; Holt et al. 2007). Bronopol (2-bromo 2-nitropropane-1.3-diol) is a widely used biocide in many industries such as pharmaceutical, cosmetic preservation, paper mills, and oil exploration due to its broad spectrum of antibacterial activity (Telang et al. 1997). Despite the success of chemical biocides, the various environmental regulatory bodies globally suggest to encourage the use and development of biodegradable and less toxic "green" biocides that will not affect other higher non-target aquatic organisms (Frey 1998). Currently, the bronopol is used for inhibition of corrosion in MS 1010. Bronopol is well-known antibacterial activity against microbes. However, the application of bronopol to control the chemical and biological corrosions is very limited. Bronopol may act as protective layer on metal surface with their functional group of nitrogen and reduced the corrosion rate. Hence, in this present study, the application of bronopol on inhibition of both chemical and microbial corrosions is explored.

Microbial corrosion studies involving the use of indigenous individual species isolated from industrial systems are scarce. However, such studies would certainly increase the understanding the interactions of the bacterial species involved in MIC with the metal surfaces/biocide to address the prevailing problem in a better way. Therefore, the present study is attempted to isolate the bacteria from cooling tower system and identify them by 16S rRNA gene sequencing. Following the molecular identification, the role of the isolates towards MIC formation on the surface of mild steel (MS) 1010 was examined and confirmed by weight loss method, electrochemical measurement (polarization and impedance), surface analysis—scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) techniques. In addition, anti-corrosive studies using an eco-friendly biocide, bronopol, was also investigated.

## Materials and methods

### Sample collection and isolation of bacterial strains

Biofilm and water samples were collected from a cooling tower water system located at a chemical company, Sipcot, Ranipet, Vellore district, Tamilnadu, India (Latitude 12.953671° N and Longitude 79.313132° E). Three biofilm samples from inlet, outlet, and inside the cooling tower system were collected into sterile sample containers and transferred to the laboratory in an ice box for further experiments. The collection of biofilm samples was performed following a standard procedure of (Rajasekar and Ting 2014). The physicochemical parameters of the cooling tower water are shown in Table 1.

The biofilm samples were serially diluted (tenfold) using sterile saline (NaCl 0.85%) and plated using a pour plate technique on different selective media, namely, American Petroleum Institute (API) medium consisting (g/L) of 1-yeast extract, 0.2-magnesium sulphate, 0.01-dipotassium phosphate, 0.1-ferrous ammonium sulphate, 10-sodium

**Table 1** Physiochemical characterization of water collected from cooling tower

| S. no | Physicochemical characteristics | Quantity |
|---|---|---|
| 1 | pH | 7.3 |
| 2 | Conductivity (μS/cm) | 1800 |
| 3 | Color | Colorless |
| 4 | Temperature (°C) | 38 |
| 5 | Turbidity (NTU) | 1.4 |
| 6 | BOD (mg/L) | 3.62 |
| 7 | COD (mg/L) | 20.17 |
| 8 | Alkalinity (600 mg/L) | 704 |
| 9 | Total Hardness (mg/L) | 240 |
| 10 | Carbonate Hardness (mg/L) | 230 |
| 11 | Chlorides (mg/L) | 660 |
| 12 | Fluorides (mg/L) | 0.5 |
| 13 | Nitrites (mg/L) | 0.04 |
| 14 | Calcium (mg/L) | 0.07 |
| 15 | Manganese (mg/L) | 0.03 |

chloride, 0.1-ascorbic acid, and 15-agar; iron oxidizing medium (IOM) consisting (g/L) of 5-casein enzymic hydrolysate, 3-yeast extract, 10-L-lysine hydrochloride, 5-mannitol, 1-dextrose, 1-salicin, 0.1-L-cystine, 0.5-ferric ammonium citrate, 0.1-sodium thiosulphate, 0.025-neutral red, and 15-agar; manganese bacterial agar (MA) consisting (g/L) of 1-beef extract, 0.075-yeast extract, 2-manganese carbonate, 0.15-ferrous ammonium sulphate, 0.15-sodium citrate, and 12-agar; and nutrient agar (NA) consisting(g/L) of 5-peptic digest of animal tissue, 5-sodium chloride, 1.5-beef extract, 1.5-yeast extract, and 15-agar. All chemicals were of analytical grade (Himedia, India) and used without further purification. Viable bacteria were counted on each sterile selective agar plates after an incubation period of 24–48 h at 37 °C.

## Molecular identification of bacteria and phylogenetic analysis

According to Bergey's manual of determinative bacteriology (Holt et al. 1994; Logan et al. 2009), the isolated bacterial cultures were identified based on their morphology, biochemical characteristics. Morphological and biochemical properties were characterized using the following tests: (i) gram staining; (ii) motility test; (iii) indole production; (iv) methyl red test; (v) Voges–Proskauer test; (vi) citrate utilization test; (vii) carbohydrate fermentation test; (viii) catalase test; (ix) oxidase test; (xi) starch hydrolysis; (xii) gelatin hydrolysis; and (xiii) a lipid hydrolysis test (Logan et al. 2009). Genomic DNA from each of the bacterial isolates was extracted following the protocol of Ahmed et al. 2007). The extracted genomic DNA was subjected to polymerase chain reaction (PCR)

amplification in a Eppendorf thermocycler, Model 5331. Amplification of the gene encoding 16S rRNA was carried out using the forward primer 5′-AGAGTTTGATCC TGGCTCAG-3′ and the reverse primer 5′-ACGGCTACC TTGTTACGACTT-3′) (Weisburg et al. 1991). PCR was performed in a 50 μL reaction mixture containing 2 μL of template DNA, 1 μL of forward and reverse primers each at a concentration of 0.5 μM, 1.5 μL of 1.5 mM $MgCl_2$, 1 μL of each dNTP at a concentration of 50 μM and 1 μL of *Taq* DNA polymerase, and 5 μL of buffer as recommended by the manufacturer (MBI Fermentas, USA). The PCR reaction was carried out under the following conditions: initial denaturation at 95 °C for 1 min; 40 cycles of denaturation (3 min at 95 °C), annealing (1 min at 55 °C), extension (2 min at 72 °C), and a final extension (at 72 °C for 5 min) followed by a hold (at 4 °C for infinity). The PCR products were purified using Montage PCR Clean up kit (Millipore, USA). The sequencing was carried out using a Big Dye terminator cycle sequencing kit (Applied BioSystems, USA), and sequences for the bacterial 16S rRNA were determined using automated DNA sequencing system (Applied BioSystems, model (3730XL, USA). Quality control of DNA sequence was using the FASTX Toolkit. This toolkit was capable of checking base quality and nucleotide distribution.

The obtained sequences were analysed with BLAST search version 2.2.20 (Altschul et al. 1990) and accessories of the Ribosomal Database Project-II Release 10 (http ://rdp.cme.msu.edu) to determine the taxonomic hierarchy of the sequences. Taxonomically associated sequences were acquired from the National Center for Biotechnology Information (NCBI) taxonomy database and Ribosomal Database Project-II (Release 10). Phylogenetic and similarity analyses were performed on 16S rRNA gene regions, and alignment gaps were treated as missing data by the MEGA software program (Version 4.1) (Tamura et al. 2007). Phylogenetic trees were constructed using neighbor-joining method and 1000 bootstrap imitations were conceded to authenticate the internal branches. Mat GAT version 2.01 was used to estimate the similarity percentages between sequences.

## Preparation of bacterial inoculum

*Bacillus thuringiensis* EN2, *Terribacillus aidingensis* EN3, and *Bacillus oleronius* EN9 were selected for the biocorrosion study. Sterile nutrient broth (NB, 100 mL) was inoculated with a loopful of culture ($1.4 \times 10^4$ CFU/ml) and incubated in an orbital shaker at 200 rpm for 12 h at 37 °C. A mixed consortium was prepared by mixing the initial inoculum of all the three isolates at a concentration of $1.4 \times 10^4$ CFU/ml.

## Preparation of biocide formulation

Bronopol (2-bromo-2-nitropropane-1.3-diol) was used as biocide for inhibition of biofilm on mild steel (MS) metal surface. The analytical grade chemical bronopol was purchased from Himedia (RM6724) and was used at different concentrations 5, 10, 15, 20, and 25 ppm to determine the minimal inhibitory concentration against corrosive bacteria.

## Biocorrosion studies

Biocorrosion of mild steel 1010 (C 0.2%, Fe 0.03%, Mn 0.2%, P 0.50%, S 0.03%, and remaining iron) was used as described by Rajasekar and Ting (2014). Briefly, MS disc coupon measured 10 mm diameter and 2 mm thickness, $50 \times 10$ mm (weight loss) and 0.1 cm$^2$ for electrochemical impedance spectroscopy (EIS), while rectangular coupons measured 2 mm thickness. The surfaces of coupons were consecutively grounded and mirror finish polished was done manually using 180, 500, 800, 1200, and 1500 grit silicon carbide paper and micro-alumina powder (0.3 µm). The coupons were rinsed with deionized water, air-dried, and subsequently immersed in 70% ethanol for 10 min for surface sterilization and stored in a desiccator prior to experiment. Both the coupons were used for biocorrosion, electrochemical, surface analysis, and weight loss studies. The polished coupons were introduced into 500 mL Erlenmeyer flasks containing 400 mL of cooling tower water was used as electrolyte. Experimental system I consisted of 400 mL of cooling tower water with 1% NB inoculated with 1 mL of EN2 ($1.2 \times 10^4$ CFU/mL); experimental systems II and III with EN3 ($1.4 \times 10^4$ CFU/mL) and EN9 ($1.8 \times 10^4$ CFU/mL), respectively, and experimental system IV, with mixed consortia. Experimental system V consists of system IV with the addition of 20 ppm of biocide, bronopol. The control system consisted of 400 mL of sterile cooling tower water with 1% sterile nutrient broth (NB) (Table 2). Three coupons were introduced into each biocorrosion system and the system experiments were carried out in triplicate.

At the end of the experiment (14th day), the coupons were removed from their respective systems, and corrosion products were carefully scraped off from coupons, and analysed by FTIR spectroscopy and X-ray diffraction (XRD) analysis (Rajasekar and Ting 2014). Soon afterwards, the coupons were pickled in Clarke's reagent (1 l HCl containing 20 g antimony trioxide and 50 g stannous chloride) at room temperature for 25 min to remove the corrosion products completely. The final weight of each coupon was estimated and the average weight loss and standard deviation (SD) were calculated. The corrosion rate was calculated as recommended by National Association of Corrosion Engineers (NACE) (Mcintyre and Mercer 2010).

## Surface analysis

The corrosion products were crushed into a fine powder before surface analysis. A computer-controlled XRD system (Bruker 8030) between 10° and 85° − 2 Θ with copper Ka radiation (35 kV/25 mA) was performed to study the nature of the obtained corrosion product. An FTIR spectrometer (Bruker's VERTEX 70 series) equipped with a narrow band of liquid nitrogen-cooled HgCdTe (MCT Optics) detector was used for IR spectral analysis (Rajasekar and Ting 2014). The surface morphology of the biofilm and the surface of the metal coupons was characterized using a scanning electron microscope (SEM) (JEOL JSM-5600LV) operated at 15 kV beam electrical energy (Rajasekar and Ting 2010).

## Electrochemical analysis

Electrochemical tests were carried out using a three electrode system (CH Instrument Inc., USA model CHI 608E). A MS coupon was employed as the working electrode, Ag/AgCl as reference electrode, and platinum wire as counter electrode. The Electrochemical Impedance Studies (EIS) were measured at open-circuit potential using a 10 mV applied amplitude and the impedances were measured for frequencies ranging from 100 to 0.01 Hz. The same MS coupons were used for polarization to measure Tafel polarization operated at an open-circuit potential towards 200 mV

**Table 2** Experimental scheme of biocorrosion system in cooling water system

| S. no | Systems | Experimental scheme |
|---|---|---|
| 1 | Control | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) |
| 2 | System I | 400 mL in sterile cooling tower water containing 1% of NB with 1 mL of *B. thuringiensis* culture ($1 \times 10^4$ CFU/mL) |
| 3 | System II | 400 mL in sterile cooling tower water containing 1% of NB with 1 mL of *T. aidingensis* culture ($1 \times 10^4$ CFU/mL) |
| 4 | System III | 400 mL in sterile cooling tower water containing 1% of NB with 1 mL of *B. oleronius* culture ($1 \times 10^4$ CFU/mL) |
| 5 | System IV | 400 mL in sterile cooling tower water containing 1% of NB with mixed bacterial consortium ($1 \times 10^4$ CFU/mL) |
| 6 | System V | 400 mL in sterile cooling tower water containing 1% of NB with mixed bacterial consortium ($1 \times 10^4$ CFU/mL) and 20 ppm biocide (bronopol) |

anodically and cathodically, at the scan rate of 120 mV/min (Rajasekar and Ting 2010).

## Extraction and quantification of extracellular polysaccharides (EPS)

Each bacterial culture broth was centrifuged (Remi, C-24BL) at 96768×$g$ for 15 min at 4 °C and the obtained supernatant was filtered through 0.2 μm mixed cellulose ester filters (Advantec, Ehime, Japan). Three volumes of ice cold isopropanol was added to the supernatant and incubated at − 20 °C overnight (Dubois et al. 1956). The secreted EPS was then centrifuged at 96768×$g$ for 15 min at 4 °C. The collected pellet was re-suspended in 1× phosphate buffered saline (PBS-137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, and 2 mM $KH_2PO_4$, pH 7.4). This partially purified EPS was used for further analyses. Extracted EPS was dissolved in sterile distilled water and subjected to protein and carbohydrate analyses. The protein and carbohydrate amounts present in the EPS were estimated using Lowry and phenol/sulphuric acid methods (Dubois et al. 1956), respectively. The purified EPS were analysed in a ultraviolet–visible (UV–Vis) spectrophotometer (UV-2450 model, Jasco Inc., Japan) in a range of 200–800 nm (Singh et al. 2012) and FTIR spectroscopy (Bruker's VERTEX 70 series), in which the EPS were mixed thoroughly with potassium bromide (KBr) and prepared KBr pellets. Then, KBr pellet samples were scanned from 4000 to 400 $cm^{-1}$ wave length range (Rajasekar and Ting 2014).

## Bacterial adhesion to hydrocarbons (BATH) assay

The BATH assay was done to measure the hydrophobicity of the isolated corrosive bacteria (Pruthi and Cameotra 1997; Bento et al. 2005). The EN2, EN3, and EN9 cells were harvested from the cultures by centrifugation at 43008×$g$ for 10 min at 4 °C. The cell pellets were collected, washed twice, and re-suspended in PBS (g/L of, $K_2HPO_4$ 22.2 g, $KH_2PO_4$ 7.26 g, urea 1.8 g, and $MgSO_4.7H_2O$ 0.2 g) at an initial absorbance of 0.12–0.14 at 400 nm. Test tubes containing 1 mL of hexadecane and 2 mL of cell suspension were vortexed at high speed for 2 min and then incubated at 30 °C for 15 min to allow hydrocarbon separation. The percentage of cell attachment to hexadecane was measured using the following formula:

Bacterial cell adhesion (%)

$$= \left(1 - OD_{\text{with hexadecane}}/ OD_{\text{cells suspended}}\right) \times 100.$$

The hydrophobicity of the isolated bacteria was evaluated by determining the emulsification index ($E_{24}$) (Zhong et al. 2007). This was done by mixing 2 mL of hexadecane with an equal volume of the cell suspension, vortexed for 2 min,

**Table 3** Biochemical characterization of *Bacillus*-related species from cooling tower water

| Characteristics | EN2 | EN3 | EN9 |
|---|---|---|---|
| Gram stain | Positive | Positive | Positive |
| Shape | Rod | Rod | Rod |
| Motility | + | + | + |
| Sporulation | + | − | + |
| Growth at | | | |
|   20 °C | − | − | − |
|   30 °C | + | + | + |
|   40 °C | + | + | + |
| Indole test | − | − | − |
| Methyl red test | + | + | + |
| Voges–Proskauer test | + | + | + |
| Citrate utilization test | − | + | − |
| Oxidase test | + | + | + |
| Catalase test | + | + | + |
| Production of acid form | | | |
|   Glucose | + | + | + |
|   Galactose | + | − | + |
|   Fructose | + | + | + |
|   Sucrose | − | + | − |
| Hydrolysis of | | | |
|   Starch | + | + | + |
|   Cellulose | + | − | − |
|   Casein | − | − | + |
|   Urea | + | + | − |

(+) positive response, (−) negative response

and incubated at 37 °C for 24 h. $E_{24}$ is defined as the percentage of the emulsified layer height (mm) divided by the total height of the liquid column (mm).

## Results

### Bacteriological analysis of cooling tower water

Biochemical tests of the corrosive isolates showed that they belong to Gram-positive group of bacteria. The biochemical characterization of each isolate is presented in Table 3. All the bacterial isolates tested showed positive results to catalase and oxidase tests. The neighbor-joining method was used to construct the phylogenetic trees (Fig. 1) to find out the correlations between the sequences of the ribosomal collection and related microorganisms from the GenBank database. The similarity and species identified with the phylogenetic analysis are given in Table 4. Briefly, *B. thuringiensis* EN2 > 99%, *T. aidingensis* EN3 > 99.2%, likewise *B. aryabhattai* EN8 > 98%, *B. oleronius* EN9 > 99%, *B. cereus*
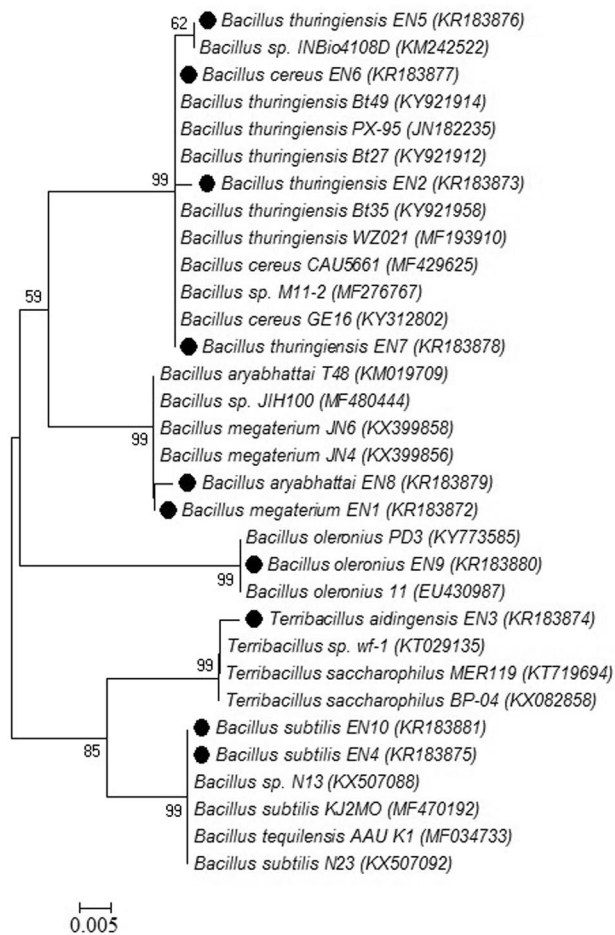
**Fig. 1** Neighbor-joining tree based on 16S rRNA gene sequences, showing phylogenetic relationships between sequences of the *Bacillus* sp. and *Terribacillus* sp. Numbers at nodes indicate bootstrap values > 50% from 1000 replicates. GenBank accession numbers are given in parentheses. The scale bar indicates sequence divergence

EN6 > 99%, *B. megaterium* EN1 > 99%, and *B. subtilis* EN10 > 99%.

## Nucleotide sequence accession numbers

The 16S rRNA sequences reported in this paper have been deposited in the GenBank database, under the following accession numbers: three isolates of *B. thuringiensis* (KR183873, KR183878, and KR183876), two isolates of *B. subtilis* (KR183875 and KR183881), *T. aidingensis* (KR183874), *B. aryabhattai* (KR183879), *B. oleronius* (KR183880), *B. cereus* (KR183877), and *B. megaterium* (KR183872).

## Biocorrosion studies

### Weight loss and Surface analysis

The weight loss data and corrosion rates calculated for MS coupons in the presence or absence of corrosive bacteria and/or biocide are presented in Table 5. In the control system, the average weight loss of the MS metal coupon is noted as 111 mg, whereas higher values of 163, 162, 140, and 286 mg are noted for individual cultures of EN2, EN3, and EN9 and mixed consortia, respectively. In case of biocide added system, a decreased average weight loss of 32 mg is noted. Consequently, a corrosion rate of about 35 mm/y is noted in the absence of bacteria, and for systems I, II, III, and IV; the corrosion rates are about 52, 51, 45, and 91 mm/year, respectively. Notably, weight loss and corrosion rate (10 mm/year) were highly reduced in the addition of bronopol (System V), and thus, inhibition efficiency was 71%.
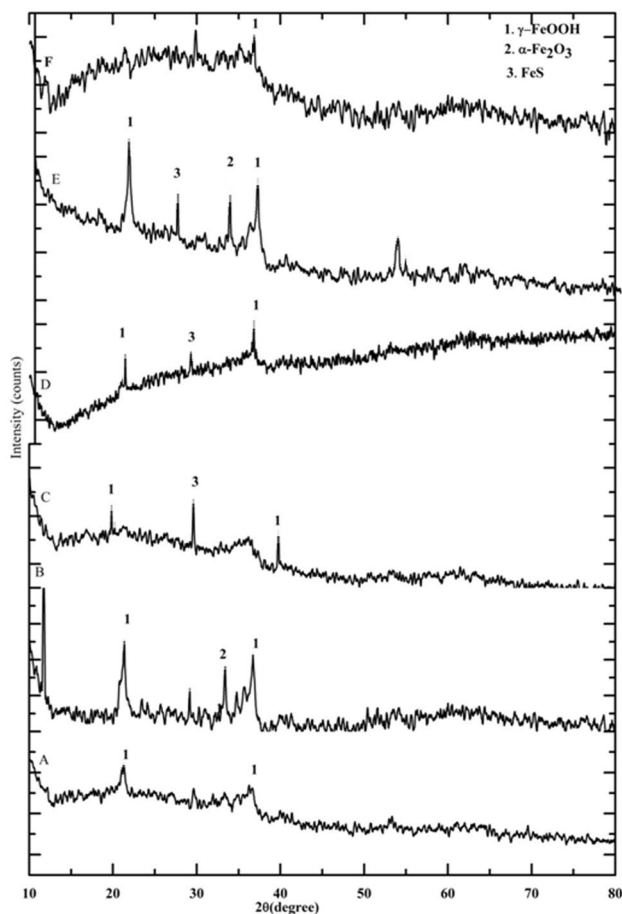
Corrosion products collected from each biocorrosion system were characterized by XRD (Fig. 2). Low intensity peak of iron hydroxide (γ-FeOOH) was detected in control system (Fig. 2a), whereas the bacterial systems (I–IV) showed the

**Table 4** 16S rRNA sequence analysis of bacterial isolates from cooling tower water

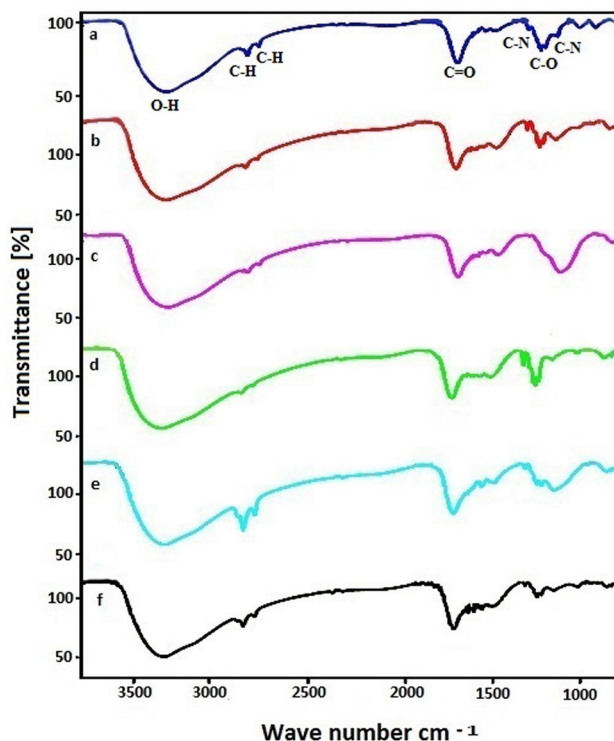| S. no | Nature of sample | Name of the sample | Identity (%) | Best match | Gene bank accession no. |
|---|---|---|---|---|---|
| | Biofilm | | | | |
| 1 | | EN2 | 99 | *Bacillus thuringiensis* | KR183873 |
| 2 | | EN3 | 99.2 | *Terribacillus aidingensis* | KR183874 |
| 3 | | EN8 | 99 | *Bacillus aryabhattai* | KR183879 |
| 4 | | EN9 | 98 | *Bacillus oleronius* | KR183880 |
| 5 | | EN5 | 99 | *Bacillus thuringiensis* | KR183876 |
| | Outlet recirculating water | | | | |
| 6 | | EN6 | 99 | | |
| 7 | | EN7 | 98 | | |
| 8 | | EN1 | 99 | | |
| 9 | | EN4 | 98 | | |
| 10 | | EN10 | 99 | | |

**Table 5** Corrosion rate of MS (1010) in the presence/absence of corrosive bacteria and biocide

| S. no | Systems | Weight loss (mg) | Corrosion rate (mm/year) | Inhibition efficiency (%) |
|---|---|---|---|---|
| 1 | Control system | $111 \pm 2$ | $35 \pm 2$ | – |
| 2 | System I | $163 \pm 1$ | $52 \pm 1$ | – |
| 3 | System II | $162 \pm 1$ | $51 \pm 2$ | – |
| 4 | System III | $140 \pm 2$ | $45 \pm 2$ | – |
| 5 | System IV | $286 \pm 2$ | $91 \pm 1$ | – |
| 6 | System V | $32 \pm 2$ | $10 \pm 1$ | 71 |



**Fig. 2** XRD spectrum of corrosion product collected from various biocorrosion systems: **a** control, **b** system I, **c** system II, **d** system III, **e** system IV, and **f** system V



**Fig. 3** FTIR spectrum of surface film on the MS surface coupons collected from various biocorrosion systems: **a** control, **b** system I, **c** system II, **d** system III, **e** system IV, and **f** system V

high intensity peaks of $\gamma$-FeOOH, iron oxide ($\alpha$-$Fe_2O_3$), and ferrous sulphide (FeS). The presence of biocide bronopol (system V) also showed the low intensity peaks as observed in the control system.

The results of the FTIR spectra of the corrosion products in the absence/presence of corrosive bacteria/biocide are shown in Fig. 3. In both control (Fig. 3a) and experimental systems (Fig. 3b–f), a wide band observed within the wave length range of 3000–3500 cm$^{-1}$ is assigned to OH group. Additional peaks observed in the range of 2950–2850 cm$^{-1}$ are assigned to CH-aliphatic group. Other absorption peaks (between 1600 and 1500 cm$^{-1}$) is attributed to the typical C–O and C–N bonds of the corrosion products. A peak at 1370 cm$^{-1}$ is assigned to CH alkanes. On contrary, the biocide system V showed low intensity peaks at 1600–1000 cm$^{-1}$ range when compared to control and experimental systems. The surfaces of MS coupons removed from the systems I–IV were subjected to SEM, and their respective images are presented in Fig. 4. The coupons in bacterial systems (Fig. 4b–e) show the dense bacterial colonies and films covering on their surfaces. However, there is no such accumulation of materials on the coupons treated with biocide (Fig. 4f). A similar result was observed in biocide and the control systems (Fig. 4a).

## Electrochemical analysis

Figure 5 shows the Tafel polarization curves for MS in a cooling tower water system in the absence and the presence of bacterial isolates (systems I–IV) and the biocide (system V). The related data are presented in Table 6. The corrosion current ($I_{corr}$) of the control system is noted as
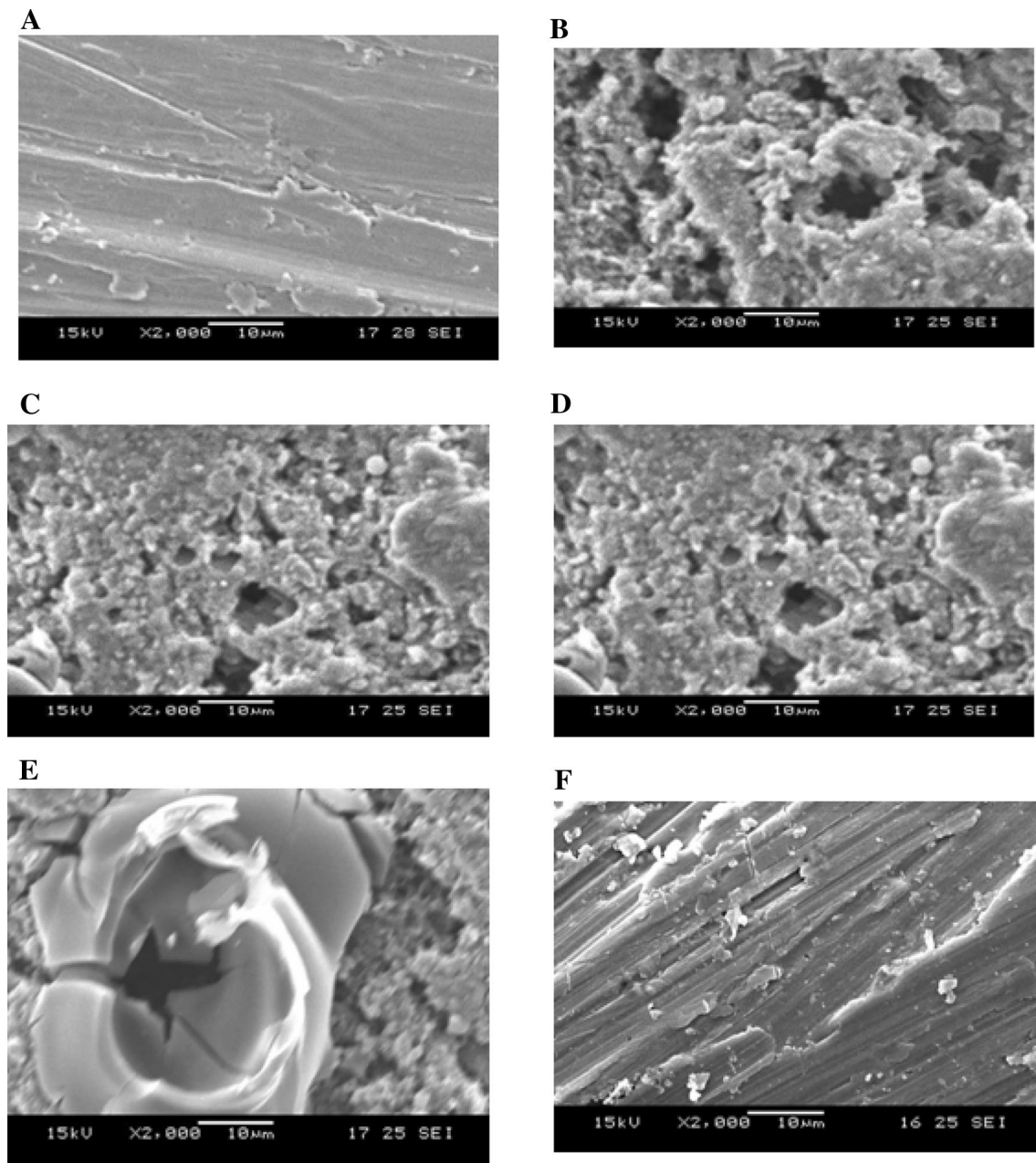
**Fig. 4** SEM micrograph of the MS Surface coupons after immersion in various biocorrosion systems: **a** control, **b** system I, **c** system II, **d** system III, **e** system IV, and **f** system V

very low (for instance $10^{-9}$) compared to the bacterial systems I, II, III, and IV which gives higher $I_{corr}$ values ($10^{-7}$ to $10^{-8}$). $I_{corr}$ value was considerably reduced in the addition of the bronopol in the bacterial system ($4.5 \times 10^{-9}$ A/cm$^2$). The Nyquist plot in the absence/presence of bacteria and biocide system is shown in Fig. 6. From the plot, the charge transfer resistance ($R_{ct}$) and solution resistance ($R_s$) were higher in presence of bronopol system ($R_{ct}$) when compared to control system ($R_{ct}$) (Table 6).

## Quantification of EPS

EPS secreted by all the three isolated strains (EN2, EN3, and EN9) were characterized for their protein and carbohydrate contents. Protein and carbohydrate concentrations of three isolates are 44, 35, and 40% and 18, 13, and 16%, respectively. The FTIR spectrum of the EPS extracted from EN2, EN3, and EN9 is shown in Fig. 7. A wide absorption band at 3439 cm$^{-1}$ and a weak absorption band at 2923 - 2853 cm$^{-1}$
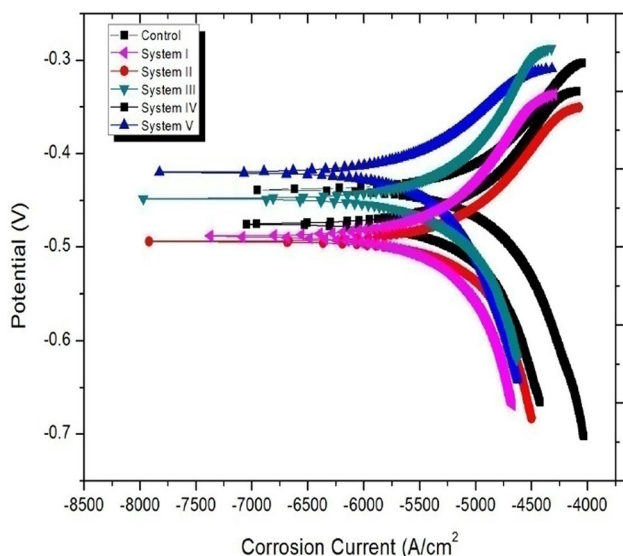
**Fig. 5** Tafel polarization curves for mild steel in presence/absence of bacteria and biocide in various biocorrosion systems

**Table 6** Polarization and impedance parameters for mild steel 1010 in the presence/absence of corrosive bacterial isolates and biocide

| System | Polarization | | Impedance data | |
|---|---|---|---|---|
| | $E_{corr}$ (mV) | $I_{corr}$ (A/cm$^2$) | $R_s$.$\Omega$ cm$^2$ | $R_{ct}$.$\Omega$ cm$^2$ |
| Control | − 523 | $4.9 \times 10^{-9}$ | 155 | 84 |
| System I | − 345 | $6.5 \times 10^{-7}$ | 92 | 31 |
| System II | − 375 | $6.1 \times 10^{-7}$ | 111 | 35 |
| System III | − 456 | $5.6 \times 10^{-8}$ | 121 | 51 |
| System IV | − 332 | $6.8 \times 10^{-7}$ | 87 | 27 |
| System V | − 562 | $4.5 \times 10^{-9}$ | 162 | 93 |

$E_{corr}$, corrosion potential; $I_{corr}$, corrosion current; $R_s$, solution resistance; $R_{ct}$, charge transfer resistance

correspond to hydroxyl group and asymmetrical C–H groups, respectively. Figure 7b shows the amide II stretching vibration and amine N–H group at 1551 and 855 cm$^{-1}$, respectively. The bacterial cell surface hydrophobicity of the three isolates (EN2, EN3, and EN9) was assessed by BATH assay. When grown in n-hexadecane containing medium, EN2 showed higher emulsification index ($E_{24}$) (80%) when compared to EN3 (60%) and EN9 (65%). These results suggest the strain EN2 as the highest biosurfactant producer than other strains.

## Discussion

*Bacillus* sp. and *Terribacillus aidingensis* were identified as the dominant group of corrosive bacteria presented in the cooling water system as described by Hussain et al.
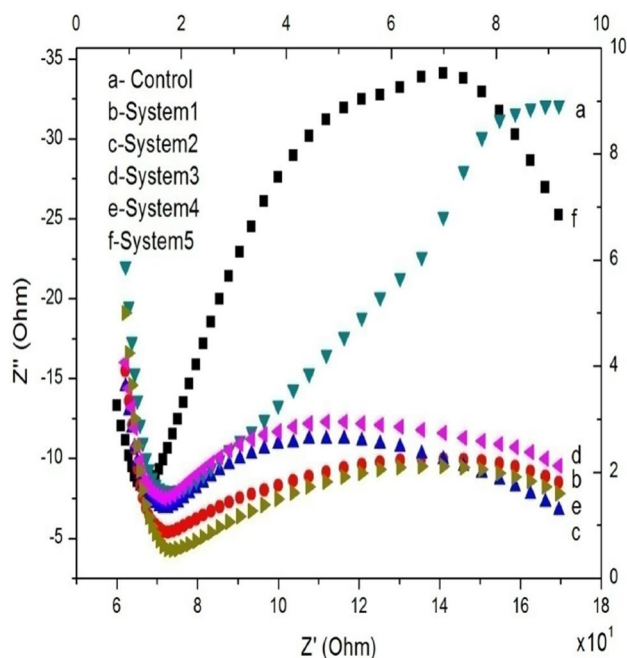


**Fig. 6** Impedance curve for mild steel in presence/absence of bacteria and biocide in various biocorrosion systems: **a** control, **b** system I, **c** system II, **d** system III, **e** system IV, and **f** system V
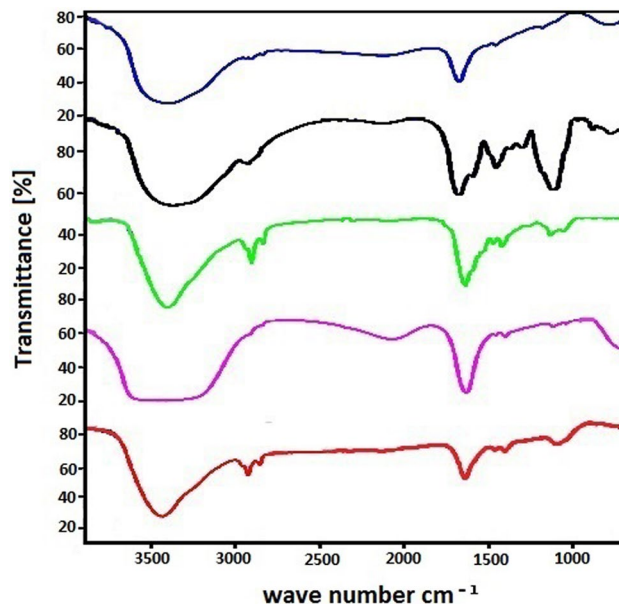


**Fig. 7** FTIR spectrum of EPS on mild steel in different biocorrosion systems: **a** system I, **b** system II, **c** system III, **d** system IV, and **e** system V

(2013). The genus *Bacillus* comprises aerobic and facultative anaerobic species, rod-shaped, and motile cells and *Terribacillus aidingensis* was moderately halophilic bacteria, rod-shaped, and motile. Since all the bacterial isolates

tested were catalase and oxidase positive, they can metabolize the substrate using oxygen as the final electron acceptor. According to Busalmen et al. (2002), bacterial production of catalase stimulates the microbial corrosion of metals by increasing the oxygen reduction current and oxidizes ferric into ferric oxide which was confirmed by formation of red color in the growth media. The species *B. thuringiensis* and *B. cereus* were previously reported to accelerate the corrosion process in cooling tower material (Bano and Qazi 2011). Thus, the identification results of the isolated strains confirmed the possible species that can accelerate corrosion.
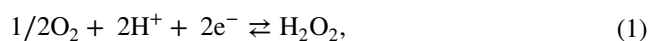
The corrosive behaviour of the isolates on the MS surface was quite obvious with the obtained electrochemical analysis and weight loss measurement studies. As seen from the results, a higher weight loss in the bacterial system is due to the utilization of the ferrous ion as inorganic source during oxidation of ferrous ion into ferric oxides (Hedrich et al. 2011; Narenkumar et al. 2016; Elumalai et al. 2017). This might have facilitated the formation of iron oxides which further react with $Cl_2$ in the corrosive environment (growth medium) (Telang et al. 1997). Thus, the final product, ferric chloride, has led to a pitting type of corrosion on the MS surface. On the other hand, a significant reduction of weight loss in the biocide system is due to antibacterial activity of bronopol, which was inhibited the biofilm formation on the MS surface, indirectly which leads to the controlling of the corrosion reactions (Rajasekar and Ting 2014). The SEM analysis also supported the CR results by confirming the capability of biofilm formation by all the bacterial isolates to accelerate the corrosion process (Stadler et al. 2008). However, a consequence reduction in CR and $I_{corr}$ of the biocide system is due to suppression of both the anodic and cathodic reactions. A higher solution resistance ($R_s$) value of the biocide system confirmed the adsorption of biocide components on the metal surface, thus forming a protective layer (a compact nitrogen band with an iron oxide layer). Bronopol was adsorbed on the MS metal surface as complex with $Fe^{3+}$ oxide and led to form a protective layer. It was also evident from the EIS analysis viz the higher charge transfer resistance ($R_{ct}$ 93 $\Omega$ cm$^2$). In the presence of bacteria, the bronopol system V, the corrosion rate was observed lesser when compared to control and bacterial system IV. This might be the inhibition of adsorption of corrosive chemicals as well as the inhibition of biofilm on the MS surface (Rajasekar and Ting 2014). The impedance results of the bacterial system showed a depressed circle, indicating the formation of non-protective porous layer on the metal surface (Ashassi-Sorkhabi and Nabavi-Amri 2002). Whereas, in the presence of biocide, a large, well-defined semicircle capacitive loop is probably due to the formation of an intact adsorbed biocide film on the metal surface. This observation has been fully corroborated by the results of polarization studies.

XRD data revealed the presence of iron and sulphide oxides in the corrosion products of all the three bacterial systems (I–IV), thus confirming the corrosive behaviour of the isolated strains. The reduced oxide peaks in the system V are possibly due to the inhibition of the bacterial biofilm formation on the metal surface by the biocide. Therefore, bronopol can be considered as an effective biocide for controlling the biocorrosion of MS 1010 in cooling water system.

Surface hydrophobicity of microorganisms is recognized as a dominant factor which influences their adhesion to metal/non-metal surface (Rajasekar and Ting 2014). Moreover, the hydrophobicity of the bacterial isolates also plays a key role in interactions between polar and slightly polar molecules, and between particles and cells in water (Van Oss 1997). Among the three isolates, the best results of hydrophobicity (80%) and emulsification index ($E_{24}$) were noted with strain EN2. This result confirmed the high production of biosurfactant molecules by this strain due to higher uptake of hexadecane hydrocarbons present in the medium. The surface hydrophobicity and emulsification index of bacterial strains confirm their adhesion capability on the metal surface which might have led to the acceleration of corrosion process. The capability of EPS to bind to metal ions is an important factor in determining the MIC behaviour of the bacterial isolates (Yun and Park 2003). The higher protein and carbohydrate concentration of EN2 has been confirmed its effective adhesion on the metal surface (Fig. 6). This is due to the high surface hydrophobicity and EPS production nature of EN2, which lead to form a compact biofilm on the metal surface.

FTIR analysis also confirmed the secretion of EPS (COO–, amide group) by all the three isolates on metal surfaces. However, system V, a significantly reduced EPS peak intensity, was due to the inhibition of biofilm formation on the metal surface. This may be due to the antibacterial activity of bronopol (Shepherd et al. 1988). The antibacterial action of the biocide can be contributed to the catalytic oxidation of thiols present in the bronopol that inhibits growth and generation of free radicals leading to cell death (Holt et al. 2007; Touati 2000). Overall, the corrosion rate was found to be higher in mixed consortium of EN2, EN3, and EN9 than the individual strains and abiotic system, thus confirming the potentiality of the consortium towards acceleration of severe corrosion of the surface of MS 1010.

During bacterial metabolism, the toxic compound, hydrogen peroxide was oxidized by the action of peroxidase enzyme, which was produced by the bacteria. Further that enzyme cleaves $H_2O_2$ into water and oxygen (Busalmen et al. 2002). The product $O_2$ has the higher affinity to the metal surface to bind $Fe^{++}$ and leads to formation of corrosion product (FeOH and $Fe_2O_3$):

$$1/2O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O_2, \tag{1}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + 2OH, \tag{2}$$

$$Fe^{3+} + 2HO \rightarrow Fe\,(OH)_2. \tag{3}$$

The mechanism of corrosion can be supported by Fenton reaction too (Touati 2000) (Eq. 2). It was inferred that formation of $Fe^{3+}$ (Eq. 2) combines with $O_2$ ions and forms Fe $(OH)_2$ or FeOOH or $Fe_2O_3$ as a corrosion product. The corrosion reaction rate from ferric to ferric oxides is determined by the bacterial metabolism (Eq. 3) which was observed in XRD (Fig. 2), FTIR (Fig. 3), and SEM (Fig. 4).

## Conclusion

The genus *Bacillus* was identified as a dominant corrosive bacterial group in the selected cooling tower water system. The mixed consortium of *Bacillus* sp. and *Terribacillus* sp. has accelerated the corrosion on MS surface. Biosurfactant produced by EN2 contributes to an increased cell surface hydrophobicity (80%) which enhanced the adhesion on the MS metal surface, thus leading to the formation of a thick biofilm. SEM, EIS, and FTIR confirmed the biofilm formation by the individual and the mixed bacterial systems on MS surface after the exposure to cooling water. Biofilm was formed on the MS 1010 as microcolonies, which subsequently caused pitting types of corrosion. Besides, the bronopol used as an antibacterial agent against corrosive bacterial strains, which formed a protective layer on the metal surface and has considerably reduced the biofilm formation and corrosion of MS. Thus, the present study has unveiled the underlying mechanism of corrosion process and the corrosion inhibition effect of bronopol allows further consideration for the development of effective corrosion inhibitor for corrosion problems in cooling water tower system.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest in the publication.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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