Evaluation of microbial agents as corrosion and scale inhibitor for industrial cooling water applications

Yanglin Hu\textsuperscript{a,b}, Chuanmin Chen\textsuperscript{a,b,*} and Songtao Liu\textsuperscript{a,b}

\textsuperscript{a} Hebei Key Lab of Power Plant Flue Gas Multi-Pollutants Control, Department of Environmental Science and Engineering, North China Electric Power University, Baoding 071003, PR China
\textsuperscript{b} MOE Key Laboratory of Resources and Environmental Systems Optimization, College of Environmental Science and Engineering, North China Electric Power University, Beijing 102206, PR China
*Corresponding author. E-mail: hdccm@126.com

ABSTRACT

In this study, 6 strains of microbial agents were investigated as environment-friendly scale and corrosion inhibitors for industrial cooling water applications. The static jar tests along with characterization methods were applied to evaluate the scale inhibition performance. Results showed that under a concentration of 240 mg/L, the \textit{Nitrobacteria}, \textit{Denitrobacteria} and \textit{Lactobacillus} agents reached high CaCO\textsubscript{3} scale inhibition efficiencies of 83, 82, and 86\% respectively. Characterization methods indicated the deposited crystals morphologies were modified and the crystals peak intensities were lowered. In addition, weight loss measurements, electrochemical measurements, surface characterization analyses were conducted to study the corrosion inhibition performances and mechanisms. It was found that at 40 °C, \textit{Bacillus cereus} agent with 200 mg/L possessed the highest corrosion inhibition efficiency of 60.11\% at 3 d, together with the second-lowest current density of 13.0 \textmu A cm\textsuperscript{-2} at 12 d. The corrosion inhibition mechanisms were attributed to biofilm accumulation and biomineralization on Q235 CS surfaces to form protective film. The results suggested microbial agents have promising potential as environment-friendly scale and corrosion inhibitors for industrial cooling water applications.

Key words: bioelectrochemistry, cooling water, corrosion inhibitor, microbial agent, scale inhibitor

HIGHLIGHTS

- The feasibility of microbial agents using as scale and corrosion inhibitors was confirmed.
- The CaCO\textsubscript{3} scale inhibition efficiency of Lactobacillus agent after 24 h activation reached 87.60\%.
- \textit{Bacillus cereus} agent displayed superior corrosion inhibition performance with a first accelerated, then inhibited, finally accelerated corrosion process.
- The scale and corrosion inhibition effect closely related to microbial metabolism.

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INTRODUCTION

Cooling water systems are used to remove extra heat from heat exchanger in industrial production, during the operation, the continuous evaporation deteriorates water quality and causes corrosion and scaling problems. Such problems pose great impact on both economic and technical perspectives, therefore, proper treatment is required to eliminate the corrosion and scaling problems in cooling water system (Gan et al. 2018).

In industry, adding chemical agents is a most common approach to control both scale deposition and material corrosion problems. Traditional scale and corrosion inhibitors such as phosphonate, chromate, and molybdate has been restricted due to secondary pollution. Environmental friendly polymer inhibitors like polyacrylic acid, polyepoxysuccinic acid (PESA) (Huang et al. 2019) and polyaspartic acid (PASP) (Gao et al. 2015) need periodically addition, which further brings potential labor costs. In this perspective, it is significant to develop new corrosion and scale inhibitors for cooling water systems with high inhibition performances, environmentally friendly, and less dosing frequency.

Microbial agents, as an exogenous bacterial population, have wide applications in fields such as microbial organic fertilizer, agricultural feed additives and fermentation production (Jin et al. 2005; Jia et al. 2021; Poveda et al. 2021). Another major application area of microbial agents is wastewater treatment, which use microbial biological metabolic process to achieve the purpose of pollutant removal. During the biological process, functional microorganisms decompose organic matter to obtain energy and produced amino acids, lipids and carbohydrates as their primary metabolites (Wang et al. 2019). Such metabolites, namely microbial product, with superior characteristics of eco-friendly, strong complexing affinities, and wide range of sources, may have the potential of being corrosion and scale inhibitors in cooling water system. Several studies have been proved microbial product own validity in scale and corrosion control. The first study of utilizing biological method on scaling control was conducted by Kawaguchi (Kawaguchi & Decho 2002), which proved the soluble extracellular polymeric substances (s-EPS) of one cyanobacterium (Schizothrix sp.) was capable of inhibiting CaCO3 precipitation. A recent study also showed the s-EPS of Bacillus cereus exhibited an 87.60% CaCO3 inhibition efficiency (Li et al. 2019a, 2019b). Except directly extracted microbial extracellular polymeric substances (EPS), bio-materials derived from microbial metabolism, like xanthan gum, alginate, and itaconic acid, also possessed certain scale inhibition capacities. The
investigations suggested negatively charged functional groups in their molecular might interact with positively charged calcium ions leading crystals distortion and scale inhibition happened (Yang & Xu 2011; Karabelas et al. 2017; Cui & Zhang 2019). Besides, microorganisms’ adhesion to metal surfaces usually accelerates corrosion rate. It has been reported some microorganisms were capable of retarding metal corrosion, namely microbiologically influenced corrosion inhibition (MICI). In the study of (Suma et al. 2019), Pseudomonas putida attached on mild steel surfaces by secreting adhered EPS and accelerating biofilm formation. The Fe-EPS and stable vivianite constituted the biopassivated layer offered mild steel a long-term corrosion. (Qu et al. 2015) found that as biofilm grew with immersion time, the corrosion behavior of cold rolled steel in the presence of Bacillus subtilis was first accelerated and then inhibited. (Wu et al. 2016) affirmed that compared with abiotic blank group, the corrosion rates of Q235 carbon steel under Desulfovibrio sp. and Pseudoalteromonas sp. di-cultures were lower. The above investigations suggested microorganisms and their metabolites have promising potential in scale and corrosion inhibition.

For now, researches of microbial products in scale and corrosion inhibition are scarce, previous studies mainly carried out in seawater environment, lacking researches in circulating cooling water environment. Therefore, the aim of this study is to develop an environmentally friendly scale and corrosion inhibitor for circulating cooling water applications. The adopted microbial agents with low cost and environmental friendly characteristics possess great potential. In this study, the scale and corrosion inhibition performances of 6 microbial agents (Nitrobacteria, Denitrobacteria, Lactobacillus, Bacillus cereus, Lactobacillus reuteri, and Pseudomonas fluorescens) were investigated. Their scale and corrosion inhibition mechanisms were discussed by surface characterization techniques.

MATERIALS AND METHODS
Preparation of microbial agent
Microbial agents used as corrosion and scale inhibitor were wettable dry powder of Nitrobacteria, Denitrobacteria, Lactobacillus, Bacillus cereus, Lactobacillus reuteri, and Pseudomonas fluorescens, the bioactive cell number in per gram of dry powder as well as their producers were shown in Table. s1.

Activation & inoculation
Before usage, microbial agents were activated by mixing with brown sugar, egg white and deionized water in a conical flask, the mixture was subsequently placed in a 37 °C water bath for 24 h before inoculation. During activation, pH variation was recorded by every 4 h to characterize microbial activity and further determine the optimum inoculation time. After, the supernatant of activating liquid was used as scale and corrosion inhibitors in this study. The photo of 6 microbial agents after activation was presented in Figure 1(a).

Static test experiments
Static scale inhibition test was adopted to evaluate the scale inhibition performance of microbial agent on the CaCO3 scale according to the National Standard of China (GB/T16632-2019) (Chinese National Standard 2019). The tested solutions were...
prepared by dissolving a certain quantity of CaCl$_2$ and NaHCO$_3$ in deionized water, and the concentration of Ca$^{2+}$ and HCO$_3^−$ is 120 mg/L and 366 mg/L respectively. The tested solutions containing different dosages of microbial agent were thermostated at 40 °C for 10 h and were subsequently filtered with medium speed quantitative filter paper. After cooled to room temperature, the concentration of Ca$^{2+}$ was titrated by EDTA standard solution and the scale inhibition efficiency ($\eta$) of microbial agent was calculated by the following Equation (1):

$$\eta = \frac{V_2 - V_1}{V_0 - V_1}$$  

(1)

where $V_0$ is the total concentration of Ca$^{2+}$ before heating, $V_1$ and $V_2$ are the concentration of Ca$^{2+}$ without and with scale inhibitors after 10 h incubation at 40 °C, respectively.

**Corrosion weight loss measurements**

RCC-II rotating coupon corrosion tester (Xinyou Instrument Factory; GaoYou, China) was used for the corrosion inhibition tests according to Chinese National Standard method (GB/T18175-2014) (Chinese National Standard 2014). CS test coupons (bought from Keli Environmental Protection Equipment Co., Ltd; Yangzhou, China) with dimensions of 50 mm × 25 mm × 2 mm were used throughout the test, the chemical composition (wt.%) of which is C, 0.14; Si, 0.12; Mn, 0.41; P, 0.02; S, 0.011; Al, 0.045, balance, Fe. The main steps were as follows: 1600 mL of simulated cooling water was added to a beaker with different microbial agents inoculated at a concentration of 200 mg/L, the beaker was immersed in a water bath at 40 °C. CS test coupons were mechanically abraded with emery papers, degreased with acetone, and rinsed with distilled water. After been dried and accurately weighed, the coupons were then immersed in the experimental solution and rotated at 75 rpm for 72 h. After the tests, the CS specimens were taken out, thoroughly rinsed with distilled water, sequentially washed with acid and alkali solution, dried and accurately weighed. Each set of experiments were repeated three times to ensure reproducibility. The corrosion rate ($v$) was calculated by Equation (2):

$$v = \frac{8670 \times (m - m_0) \times 10}{s \times \rho \times t}$$

(2)

$m$ is the coupon weight loss (g); $m_0$ is the coupon weight loss in the acid cleaning test (g); $s$ is the coupon surface area (cm$^2$); $\rho$ is the coupon density (g/cm$^3$); $t$ is test time (h); 8760 is a constant which represents the hours in a year ($365 \times 24$ h); 10 is corresponding millimeters (mm/cm); the corrosion inhibition efficiency ($X$) was calculated using Equation (3):

$$X = \frac{v_0 - v_1}{v_0}$$

(3)

where $v_0$ and $v$ are the corrosion rates in the absence and presence of inhibitors, respectively.

**Electrochemical measurements**

The RCC-II tester was conducted as the platform for 12 days of electrochemical experiments. Electrochemical measurements were carried out in a CHI760E (produced by Shanghai Chenhua) electrochemical workstation. A three-electrode system including a working electrode, an auxiliary electrode, and a reference electrode were used, in which the Q235 CS coupons clamped with a PTFE platinum plate electrode holder served as the working electrodes. The operating parameters were the same as weight loss measurements. Saturated calomel electrode and platinum electrode were used as a reference and counter electrodes, respectively. To ensure stability, EIS was obtained at the end of open circuit potential (OCP) measurement by applying an alternating voltage of 5 mV over frequencies ranging from 0.01 to 105 Hz. The polarization curves were measured at the scan rate of 1 mV/s and the potential applied was in the range of −500 and 500 mV with respect to OCP.

**CHARACTERIZATION**

**Characterization of scale deposits**

Based on the results of static tests, the CaCO$_3$ precipitation in the absence or presence of 4 antiscaling microbial agents were collected, washed, dried, and then characterized by SEM and XRD to explore the scale inhibition mechanisms.
Surface analysis for corrosion inhibition

According to the results of weight loss and electrochemical measurements, the Q235 coupons after 3 days of weight loss measurements with *Bacillus cereus* and *Pseudomonas fluorescens* agents were taken out, washed with distilled water and dried in air at room temperature. The corrosive morphologies were recorded using a scanning electron microscope. Simultaneously, to further analysis the component of corrosion product, the surface layer was carefully scraped with a sterile spatula from another parallel specimen and examined with X-ray photoelectron spectroscopy (XPS, Thermo VG, USA).

RESULTS AND DISCUSSION

pH variation of microbial agents’ activated solution

The pH variation during the microbial agents activation process under 37 °C was shown in Figure 1(b). In general, pH initial values differed by microbial agents’ species, the pH variation tendency was approximately identical accompanied by significantly decline throughout the whole 36 h activation period. During the first four hours of activation, pH of most microbial agents increased slightly while pH of *Pseudomonas fluorescens* and *Lactobacillus reuteri* agents showed dramatic decline from 6.05 to 5.71 and 6.96 to 4.21, respectively. This may due to powder carrier brought organic acid and other acidic materials gradually dissolved as the activation proceeded (Li *et al.* 2012). Later, microbial agents’ pH continuous declined during 4 to 24 h of activation, demonstrating that dormant bacterial cells were gradually awakened, their metabolism produced a variety of organic acids which increased the acidity of the activation solutions. After a sharply decrease, pH of microbial agents reached low bottom and kept a steady state between 20 and 24 h of activation, this indicated bacterial cells were in a stable growth and metabolism phase. In the last activation of 24 to 36 h, most microbial agents remained stable, yet, pH levels of *Nitrobacteria* and *Denitrobacteria* agents increased from 3.85 to 4.45 and 3.79 to 4.35, respectively. This can be explained by ammonia release corresponded microbial activity decrement (Gigliotti *et al.* 2012). Hence, according to the pH variation, this study chose 24 h activated microbial agents as inoculum for further scale and corrosion inhibition experiments.

Scale inhibition performance against calcium carbonate

Comparison scale inhibition under different activation time

The activation time is an important factor as discussed above, thus, to further verified the correctness of determination on inoculating time, the CaCO₃ scale inhibition performance of 6 microbial agents after 12 h activation was compared with that after 24 h activation. The results were showed in Figure 2(a). As shown in Figure 2(a), under a concentration of 240 mg/L, the scale inhibition performances of microbial agents activated 24 h improved in varied degrees contrasted with that activated 12 h. The *Lactobacillus* agent displayed the highest improvement in scale inhibition after an additional 12 h activation, from 20% to 86%, followed by *Nitrobacteria* and *Denitrobacteria* agents, from 36% to 83% and 58% to 82% respectively. Beyond that, the additional 12 h activation had weakened impact on *Bacillus cereus* and *Pseudomonas fluorescens* agents, which presented slight increasement on scale inhibition efficiency. The scale inhibition efficiency of

Figure 2 | (a) Comparison the impact of activation time (12 and 24 h) on the scale inhibition efficiency of microbial agents. (b) Effect of microbial agents’ dosage on the CaCO₃ scale inhibition efficiency.
Nitrobacteria agent maintained unchanged between activated for 12 and 24 h. Considering the pH values of the Nitrobacteria agent were almost constant during the corresponding activation period, it can be speculated that the microbial growth and metabolism remained in relatively stable states. Therefore, the additional 12 h activation time did not contribute to its scale inhibition performance.

The comparison of scale inhibition of microbial agents after different activation time (12 and 24 h) combines with pH changes results in Figure 1(b). We speculated that microorganisms gradually recovered from dormancy after activation, they generated organic acids through metabolic activities that lowered the pH of the surrounding environment. pH values before and after 12 h activation still showed a decreasing trend, indicating microorganisms are still in the state of adaptation to the environment. However, at around 24 h activation, the pH values were lower and remained relatively stable, suggesting there’s a deeper augmentation of organic acids produced by microbial activities. These organic acids were the main forces in the scale inhibition process (Mao et al. 2018). Thus, the 24 h activation was selected as the optimum inoculating time for microbial agents.

**Effect of dosage**

After activated 24 h, bacterial suspension of microbial agents was applied to investigate the dosage effects on calcium carbonate scale inhibition efficiency, the results were plotted in Figure 2(b). As can be seen, in measured dose range, scale inhibition efficiency gradually increased, yet, a certain degree of variation still existed between different species of microbial agents. Among 6 tested microbial agents, Lactobacillus reuteri agent showed almost no scale inhibition while Bacillus cereus and Pseudomonas fluorescens agents showed slightly increased scale inhibition efficiencies from about 30% to 50% with dosage increase from 80 to 240 mg/L. Moreover, Nitrobacteria and Denitrobacteria agents owned extraordinary performances in against CaCO₃ formation, their scale inhibition efficiency increased dramatically from 7% to 83% and 5% to 82% with respect to experimental dose range. The optimum antiscaling performance appeared on Lactobacillus agent, which exhibited a remarkable antiscaling increment from 26% to 86% between the dosage of 120 and 240 mg/L.

The scale inhibition effect can be explained that multiple organic acids, proteins, and peptides secreted during microorganisms’ metabolism complexed with free calcium ions to inhibit the formation of calcium carbonate (Li et al. 2019a, 2019b). In addition, typical functional microorganisms such as Lactobacillus was capable of producing lactic acid to bind with calcium ions (Lv et al. 2021), nitrification of Nitrobacteria conversed ammonium nitrogen to oxidized N (nitrite and nitrate) could also cause a decline in pH, making Ca⁴⁺ free. All these can achieve the effect of CaCO₃ scale inhibition (Zhao et al. 2020).

**Characterization of CaCO₃ scales**

In order to reveal the underlying scale inhibition mechanisms, the surface morphology of precipitated CaCO₃ scales in the presence of 4 antiscaling microbial agents were further investigated by directly observation using SEM (Figure 3). As shown in Figure 3, contrast with blank CaCO₃ crystals with regular rhombohedral form and glaze surfaces (Figure 3(e) and 3(e')), CaCO₃ crystal formed under microbial agents became irregular with rough surfaces, indicated microbial agents had an impact on CaCO₃ crystalline morphology (Figure 3(a)–3(d)). In addition, CaCO₃ crystals morphologies differed by different strains of microbial agents, with the addition of Nitrobacteria and Denitrobacteria agents, CaCO₃ crystals surfaces became cratered with fractured defects, the number of crystalline step edges increased and the morphology showed a tendency of spherulitic shape (Figure 3(a), 3(a'), 3(b) and 3(b')). Besides, CaCO₃ crystals morphologies changed into elongated dumbbell shape with rough surfaces under the presence of Lactobacillus agent (Figure 3(c) and 3(c')), while the CaCO₃ crystals formed in overgrowth toward sequential direction under Pseudomonas fluorescens agent (Figure 3(d) and 3(d')).

The observed phenomena concluded that adding microbial agents significantly changed the CaCO₃ crystal morphology, either leading a lattice distortion or altering the crystal growth orientation, this can be explained by EPS secreted during microbial metabolism contains abundant organic substances (Sheng & Yu 2006), which capable of occupying the active growth site of calcite crystal through complexation between the functional groups (carboxyl groups (-COOH), hydroxyl groups (-OH), sulfonic groups (-SO₃)) and free calcium ions (Zhuang et al. 2018). What’s more, it was found that in biominalization, some specific amino acid and proteins play an important role in controlling CaCO₃ nucleation and crystallization, resulted in a preferential crystals growth or a spherulitic crystals morphology (Kong et al. 2018; Wada et al. 2018).
Figure 4 showed the XRD spectrum of CaCO₃ crystals precipitated under blank condition and in the presence of 4 antiscale-
ing microbial agents. The diffraction peaks at 29.24° (104), 35.89 (110), 39.33 (113), 43.07 (202), 47.43 (018), and 48.41(116) corresponds to calcite crystal, indicated that scale deposition with and without microbial agents were mainly calcite form. Moreover, adding microbial agents (Nitrobacteria and Lactobacillus) slightly reduced the intensities of characteristic peaks.

Figure 3 | SEM of CaCO₃ scales formed in the presence of microbial agents with a concentration of 240 mg/L: (a) and (a’) Nitrobacteria; (b) and (b’) Denitrobacteria; (c) and (c’) Lactobacillus; (d) and (d’) Pseudomonas fluorescens; (e) and (e’) Blank sample.
at 29.24°, indicated scale inhibition was mostly taken place in (104) plane (Figure 4(a) and 4(c)), such results were in line with (Elkholy et al. 2018). Besides, the spectrum also detected that CaCO₃ deposited with Nitrobacteria agent showed diffraction peaks at 31.61° and 45.33°, corresponding to halite crystal surface of (200) and (220) respectively (Figure 4(a)). This can be explained by ion exchange between biomolecules of extracellular polymeric substance (EPS) and solution. The antiscaling mechanisms on CaCO₃ can be explained by biomolecules of EPS such as, proteins, humic acids, and polysaccharides released Na⁺ while offering the binding sites to free Ca²⁺ to facilitate the formation of EPS–Ca²⁺ complexes (Li & Yu 2014).

Corrosion inhibition of microbial agents

Corrosion weight loss measurements

Corrosion inhibition efficiency of microbial agents with the concentration of 200 mg/L was performed by weight loss measurements. Table. s2 illustrated the corrosion rate (v) and inhibition efficiency (η) of Q235 CS after immersing in simulated cooling water for 72 h. The corrosion situation of blank sample with no microbial agents added was rather serious, of which the CS corrosion rate was 0.7243 (mm/a). However, addition of microbial agents showed a certain corrosion inhibition. Among the 6 tested microbial agents, Bacillus cereus agent possessed the maximum corrosion inhibition efficiency of 60.11% along with the lowest corrosion rate of 0.2889 mm/a, followed by Lactobacillus reuteri agent of 0.4074 mm/a corrosion rate and 43.75% inhibition efficiency. From the above results, it was concluded that microbial agents possessed a certain protective effect on Q235 carbon steel corrosion in simulated cooling water system.

EIS study

To further investigated the Q235 CS corrosion with the presence of microbial agents, EIS measurements were conducted. Figure 5 presented the EIS spectra of Q235 CS during 12 days of rotatory tests with different microbial agents and without microbial agents, time-dependent Nyquist plots are shown in Figure 5(a)–5(g) and the corresponding Bode plots are displayed in Figure 5(a’–)5(g’). Generally, a relatively larger diameter of Nyquist plot in biotic medium represents a higher corrosion resistance. As shown in Figure 5, diameter of Nyquist plot obtained in abiotic medium displayed continuous downward trend, validating an increasing corrosion impact on rotatory coupon. However, Nyquist plot in biotic medium followed another tendency during whole 12 days rotatory tests, the relatively larger diameter first decreased at initial 2 days, then
gradually increased to reached a maximum value on day 7, indicating adding microbial agents introduced a corrosion inhibition effect. Such effect was attributed to attachment and colonization of microorganisms, the microbial metabolically secreted organic molecules adsorbed on the Q235 CS surface and decreased the corrosion rate. Afterwards till day 12, the diameter became narrowed and even smaller than initial value, suggested destruction of protective layer occurred overtime which possibly due to exfoliation of biofilm-corrosion product complex or local defect (Suma et al. 2019).

Corresponding equivalent circuit (EC) was used to describing the corrosion process in Figure 5(h), corresponding electrochemical parameters of the EC are tabulated in table. As shown in Figure 5(h), the $R_s$ and $R_{ct}$ means the solution resistance and charge transfer resistance, respectively. $C_f$ represents the capacitances of the biofilm and a constant phase element (CPE), $Q_{dl}$, is introduced to represent electrical double layer (EDL), the mathematical constant relates to the surface roughness, $n$, represents deviation from ideal capacitive behavior (Qu et al. 2015).

As shown by quantitative fitting parameters in Table. s3, $R_s$ in medium with microbial agents was different from blank sample and showed undulation over whole tested period, indicating dosed microorganisms could affect the corrosion conductivity. In addition, variation of $R_{ct}$ reflects the corrosion status of Q235 CS (Liu et al. 2021). $R_{ct}$ of Q235 CS without microbial agents gradually decreased with immersion time, demonstrating a decline in electron transfer resistance and accelerated corrosion rate. Besides, $R_{ct}$ values under 200 mg/L concentration of activated microbial agents were magnitude higher, means that microbial agents obviously protected Q235 CS from corrosion.

Despite different strains were the microbial agents, their $R_{ct}$ values exhibited the same tendency during the whole experimental period, it decreased within the first tested 2 days, then significantly increased from day 4 to reached a peak value in day 7, corresponding a first accelerated, then inhibited corrosion process. This phenomenon can be explained as follows: After inoculation, the addition of electrolyte caused a slight increase in corrosion rate. Simultaneously, microbes gradually
acclimatized to the environment, they adhered on metal surface and formed inhibitive biofilm to hinder the contact between metal surface and corrosive media (Liu et al. 2021). Afterwards, \( R_{ct} \) values were then rapidly reduced in the remaining test indicating that longer run operation leads to lose protective function of complexed film, such results showed good consistency with Nyquist plot (Mehra et al. 2021a, 2021b). The overall results provided a promising method of utilizing microbial agents in Q235 CS corrosion protection.

**Potentiodynamic polarization test**

Figure 5(i) represented the Tafel polarization curves of Q235 CS coupons after 12 days of rotation tests with and without microbial agents. As can be seen, 200 mg/L dosage of microbial agents significantly lowered the current density compared with blank group, suggesting that microbial agents performed a corrosion inhibition effect on Q235 CS. The electrochemical parameters obtained from Tafel analysis including corrosion potential, \( E_{corr} \), corrosion current density, \( i_{corr} \), anodic Tafel slope, \( \beta_a \), cathodic Tafel slope, \( \beta_c \), are presented in Table 4.

As shown in Table 4, the variation on corrosion potential of Q235 CS varied as different strains of microbial agents. Compared with blank sample, \( E_{corr} \) of Nitrobacteria, Denitrobacteria, Lactobacillus, and Bacillus cereus agents shifted toward positive while \( E_{corr} \) of Lactobacillus reuteri and Pseudomonas fluorescens agents showed more negatively. Furthermore, inoculation of microbial agents resulted in a markedly decrement on corrosion current density. The \( i_{corr} \) value of Q235 CS coupons after 12 days rotary test with Pseudomonas fluorescens agent was 10.6 \( \mu \)A cm\(^{-2} \), which was the lowest value among the tested microbial agents. Followed by Bacillus cereus agent, possessed a second lowest \( i_{corr} \) of 13.0 \( \mu \)A cm\(^{-2} \). Other microbial agents including Nitrobacteria, Denitrobacteria and Lactobacillus agents also showed relatively lower \( i_{corr} \) values. Lactobacillus reuteri agent possessed the maximum \( i_{corr} \) value of 40.1 \( \mu \)A cm\(^{-2} \) among the tested microbial agents, but this value was still lower than the blank group. The analysis of potentiodynamic polarization curves were in line with EIS data above, demonstrating that microbial agents were able to protect CS from corrosion.

**Surface morphology of Q235 CS**

The pristine Q235 CS coupon along with corrosive morphologies after 3 days of corrosion weight loss measurements with and without microbial agents were presented in Figure 6. Contrasting with a smooth and shiny surface displayed by pristine CS that haven’t been immersed (Figure 6(d)), the tested samples suffered different degrees of erosion with corrosion product on their surfaces. The most serious cases appeared on blank sample, on its surface, the black iron oxide corrosion areas surrounded with yellow-brown rust were observed (Figure 6(c)). However, the presence of 200 mg/L microbial agents brought a certain protection, the Bacillus cereus agent gave coupons a homogeneous surface morphology with only speckled corrosive pits existed while coupons with Pseudomonas fluorescens agents possessed inhomogeneous rust coverage (Figure 6(a) and 6(b)). Thereby, it is obvious that Bacillus cereus and Pseudomonas fluorescens agents can enhance corrosion inhibition effect on Q235 CS coupon.

To further analysis the corrosion inhibition effect of microbial agents, two areas were boxed and elected from coupons after 3 days of immersion (in Figure 6) to represent typical Q235 CS corrosion morphologies. Blue and red boxes represent mild and deep corrosive region respectively, the surface morphology was characterized with SEM and the results were displayed in Figure 6(e)–6(g): SEM images of red boxed area, (e')–(g'): SEM images of blue boxed area in the above photos.

As shown in Figure 6, morphologies of corrosion products varied from different selected regions. In blue boxed area of biotic medium, the corrosion product showed different dimensions of enlarged rectangular crystal deposition, the depositions were closer to needle-like under Pseudomonas fluorescens agent and turned to a rhombic-diamond morphology under Bacillus cereus agent. Contrastively in abiotic medium, the deposition was irregular with stony-like morphology. For red boxed area of biotic medium, the corrosion products were multilayered and heterogeneous, the fluffy cauliflower-like structures were observed on coupons exposed to biotic medium. In abiotic medium, a high number of cracks in the background layer overlap with scattered flowery structures were observed, indicating severe corrosion damage had been implemented.

**Corrosion products analyses**

XPS spectrum was conducted to validate the composition of complex corrosion product on Q235 CS coupons after 3 days of corrosion weight loss immersion. The surface survey spectrums were shown in Figure 7 and the corresponding atomic percent of each main peak were given in Table 5. It was found that under the presence of microbial agents, the proportions of C and
N which represent the organic constituents slightly increased, while the proportion of Fe which attributed to corrosion level was decreased, indicating the presence of *Bacillus cereus* and *Pseudomonas fluorescens* agents reduced the Fe release and increased the proportion of organic matters.

Figure 6 | Photos of Q235 CS coupons surfaces before and after 3 days rotatory tests. (a): 200 mg/L *Bacillus cereus* agents; (b): 200 mg/L *Pseudomonas fluorescens* agents; (c): Blank sample; (d): Pristine CS before immersion. (e), (f), (g): SEM images of red boxed area, (e'), (f'), (g'): SEM images of blue boxed area in the above Photos.
The high resolution of Fe 2p was detailed in Figure 7(d–7(f) and O 1 s spectra was detailed in Figure 7(d’–7(f), respectively. As displayed in Figure 7(d–7(f), Fe 2p spectra was deconvoluted into four peaks. In the presence of microbial agent, the peak located in 710.3 eV represents FeO while in absence of microbial agent, the peak of 707.7 eV represents metallic iron (Fe0) species (Njoku et al. 2021). Peaks at 711.3 eV, 711.4 eV and 710.5 eV are associated to ferric oxide/hydroxide species such as Fe2O3, Fe3O4 and FeOOH while high binding energy of peaks at 713.4 eV, 713.6 eV and 712.6 eV attribute to FeSO4 (Bouanis et al. 2009; Wu et al. 2014), the peak at around 719 eV is ascribed to the satellite of Fe3+ and peaks appeared at 724 and 725 eV are α-Fe2O3, Fe3O4 and FeOOH with respect to Fe 2p1/2 (Pandarinathan et al. 2014).

In the deconvoluted O 1 s spectra (Figure 7(d’), 7(e’) and 7(f’)), the peaks located at 529.8 eV, 529.9 eV and 529.7 eV in three tested sample may be assigned to iron oxide (Mehta et al. 2021a, 2021b) and the peaks at 531.3 eV, 531 eV and 531.3 eV are the binding energy of the O2− and OH− ions, corresponding the iron oxide/hydroxide layer as detected in the Fe 2p1/2 spectrum (Boumhara et al. 2015). Peaks at high binding energy of 532.5 eV, 532.6 eV and 532.8 eV are associated to the contributions of organic oxygen in single bond of C-O (Boumhara et al. 2015), which may be the result of microbial metabolism. Besides, the peak observed at 530.6 eV for Bacillus cereus group may relate to the presence of CaO, peak located at 531.7 eV for Pseudomonas fluorescens group may relate to CaSO4. As for blank sample, both two components were found corresponded to peak at 530.5 and 532 eV respectively (Ghods et al. 2011; Lu et al. 2017; Vanthana Sree et al. 2020).

**Scale and corrosion inhibition mechanism of microbial agent**

Scaling and corrosion problems are serious threat to power plant safety operation, for eliminating the environmental pressure caused by chemical inhibitors. In our study, microbial agents were conducted as scale and corrosion inhibitors and its application feasibility were confirmed. The schematic illustration was described in Figure 8.

Microbial agents of *Nitrobacteria, Dentitrobacteria* and *Lactobacillus* played an important role in calcium carbonate deposition inhibition. During the activation process, microorganisms gradually recovered, their metabolism could promote the...

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**Figure 7** XPS surface survey scan of Q235 CS corrosion after 3 days of weight loss tests with *Bacillus cereus* agent (a); *Pseudomonas fluorescens* agent (b), and without microbial agent (c). Corresponded High resolution XPS spectrum *Bacillus cereus* agent (d) and (d’); *Pseudomonas fluorescens* agent (e) and (e’) and without microbial agent (f) and (f’).
accumulation of organic acids, the lysis of bacterial biomass also contributed to generation of large amount dissolved organic matter. Such organic matters contain abundant negatively charged functional groups such as O-H and N-H groups, amide group, carboxylic groups, and C-O-C. Therefore, they chelated with free Ca\(^{2+}\) to further reduce precipitation-forming Ca\(^{2+}\), or adsorbed on grow sites of micro-nuclei and prohibited the nucleation process (Li et al. 2019a, 2019b). Moreover, highly electronegative charged molecules of EPS could occupy grow sites to interfere with regular scale crystal lattice, result in a distorted crystal structure with modified crystal morphology rather than regular oriented calcite growth without scale inhibitor (Shen et al. 2012).

In addition, microbial agents of Bacillus cereus also inhibited Q235 CS corrosion process in simulated cooling water system. Initially, the activated microorganisms started to adapt the environment and secreted organic acid, some adhered on CS surface, given an accelerated corrosion rate along with a reduced radius of the Nyquist plot in EIS spectra. Subsequently, the attached bacteria on CS surface colonized and formed compact biofilm, the covered biofilm could produce a microenvironment between metal/biofilm interface What’s more, sessile bacterial under biofilm consume oxygen for respiration to impede the oxygen cathodic reduction and prevent its diffusion to metal surface (Khan et al. 2020), therefore the diameter of Nyquist plot became larger and the corrosion process was inhibited. Furthermore, Bacillus cereus agent also induced mineralization since a variety of microbials have been reported able to induce calcium precipitation (Han et al. 2016). As indicated by XPS spectrum, the metabolically secreted exopolysaccharides could absorb Ca\(^{2+}\) to serve as the nucleation site of calcium carbonate, such complex biomineralized film acted like a barrier to prevent CS contact with corrosive medium. The final loss of CS corrosion protection corresponded the death of functional microorganism and the detachment of cells from biofilm due to starvation (Rochex & Lebeault 2007).

**CONCLUSION**

This study proved the feasibility of using microbial agents as scale and corrosion inhibitors with environmental-friendly and cost efficient. At 40 °C, activated microbial agents showed excellent scale inhibition performances, 240 mg/L of Lactobacillus agents exhibited the maximum CaCO\(_3\) scale inhibition efficiency of 86%, Nitrobacteria and Denitrobacteria agents also showed high scale inhibition efficiencies of 83 and 82% respectively. The SEM and XRD results showed that under the presence of microbial agents, the calcite crystals morphologies were significantly modified from regular rhombohedral to rougher and more disordered.

In addition, microbial agents also exhibited obvious corrosion inhibition effect on Q235 CS, the most prominent corrosion inhibitor was Bacillus cereus agent, which showed 60.11% corrosion inhibition efficiency under 200 mg/L by 3 d weight loss.
measurements. In the following 12 d of electrochemical tests, the *Bacillus cereus* agent group showed a weak current density of 13.0 μA cm⁻², which is one order of magnitude lower than blank group. Surface analysis indicated that biofilm colonized by microbial aggregation, mixed with biomineralization barrier and corrosion product, may take the main responsibility of corrosion inhibition mechanisms. The results showed microbial agents have a promising potential as corrosion and scale inhibitors for industrial cooling water applications.

**DATA AVAILABILITY STATEMENT**

All relevant data are available from an online repository or repositories.

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