



The influence of the marine *Bacillus cereus* over carbon steel, stainless corrosion, and copper coupons

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Abstract

The present study evaluated the influence of the marine bacteria *Bacillus cereus* Mc-1 on the corrosion of 1020 carbon steel, 316L stainless steel, and copper alloy. The Mc-1 strain was grown in a modified ammoniacal citrate culture medium (CFA.ico-), CFA.ico- with sodium nitrate supplementation (NO₃-), and CFA.ico- with sodium chloride supplementation (NaCl). The mass loss and corrosion rate were evaluated after the periods of 7, 15, and 30 days. The results showed that in CFA.ico- and CFA.ico- medium added NO₃- the corrosion rates of carbon steel and copper alloy were high when compared to the control. Whereas the medium was supplemented with NaCl, despite the rates being above the averages of the control system, they were considerably below the previous results. In general, the corrosion rates induced by Mc-1 on 316L coupons were below the results compared to carbon steel and copper alloy. When analyzing the corrosion rate measurements, regardless of the culture medium, the corrosion levels decreased consistently after 15 days, being below the levels evaluated after 7 days of the experiment. Our analyses suggest that *B. cereus* Mc-1 has different influences on corrosion in different metals and environmental conditions, such as the presence of NO₃- and NaCl. These results can help to better understand the influence of this bacteria genus on the corrosion of metals in marine environments.

Keywords *Bacillus cereus* · Biocorrosion · Carbon steel · Stainless · Copper

Introduction

Metal alloys are materials commonly used in marine engineering and industrial installations, as well as in the construction of pier structures, ships, and bridges close to the marine environment. As marine environments are very conducive to corrosive processes, such as corrosion influenced by chemical and microbiological products, the safety and conservation of these structures are of great global concern (Dang and Lovell 2015). Metal corrosion can be characterized as a spontaneous process, in which the transfer of electrons from zero valence metal to a final acceptor

occurs. For this process to happen, an electron donor site, or “anode site”, needs to transfer electrons to an “acceptor site” or “Catholic site” (Gadd 2004). This coupled reaction, dependent on each other, is also known as redox, and the transfer between the two sites is always from the most negative to the most positive potential. As a result, the transfer of electrons from one site to another will result in the dissolution of the anodic site or in the corrosion itself (Hamilton 2003; Watanabe et al. 2009). The deterioration of metallic and non-metallic materials by the corrosion process is a problem widely present in many sectors of the industry, as well as enormous impacts on public safety and the environment (Muyzer and Stams 2008; Hamilton 2003). Economic losses from corrosion are about US\$ 2.5 trillion, equivalent to about 3.4% of the global Gross World Product (GDP) (Koch et al. 2016). The damages resulting from metallic corrosion affect several structures used in all productive sectors of the economy. However, the values associated with corrosion are not limited to damage directly caused by corrosion to the metal. Costs related to the prevention, an inspection of structures, loss of productivity due to interruption due

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to defects caused by corrosion also add to the total costs (Procópio 2020b).

Microbiologically influenced corrosion (MIC) is also a problem related to damage to the metallic infrastructure, especially in the marine environment, with losses that can reach billions of dollars (Koch et al. 2016). MIC on metallic surfaces is an interfacial process involving interactions between microbes, the environment, and metallic structures. Normally for this to occur, planktonic cells adhere to the metallic surfaces, called pine cells, then the process of biofilm formation begins, where there is the participation of different microbial species, which create a favorable environment for corrosion to occur (Procópio 2020b). The scientific literature describes corrosion as frequently associated with the activity of sulfate-reducing bacteria (SRB) and thio-sulfate-reducing bacteria (TRB), which are present inside the biofilm, at the biofilm–metal interface, under anaerobic conditions (Boudaud et al. 2010). More recently, attention has been directed to corrosive processes, especially metal corrosion, due to the presence of oxidizing Fe lithotrophic bacteria (FeOB) and heterotrophic bacteria, such as strains of *Bacillus* and *Pseudomonas* (McBeth and Emerson 2016; Marty et al. 2014; Lee and Newman 2003; Maia et al. 2019).

Despite the fact that Fe (II) is their primary energy source and they are often identified in corrosion biofilms, the role of the FeOB group in the MIC is poorly described and our understanding of the role of FeOB in the marine MIC is largely undervalued (Mumford et al. 2016). Studies have shown that FeOB is able to colonize and grow in steel coupons introduced into the environment (Dang and Lovell 2015; McBeth et al. 2011) or are present in corroded steel associated with ALWC structures (Marty et al. 2014). These microorganisms related to iron oxidation, accelerate the process through mechanisms such as pH change, acid production, secretion of corrosive metabolites, and extracellular enzymes (Little et al. 2007; Kato 2016). The basis of the aerobic corrosion reaction is characterized by the electrochemical coupling between the oxidation of iron (anodic reaction) and the reduction of oxygen (cathodic reaction) (Lee and Newman, 2003). The product of this reaction is ferrous iron Fe (II), which is later oxidized to ferric iron, Fe (III) (Enning and Garrelfs 2014). Fe (III) acts as a final electron acceptor in the electron transport chain, allowing the flow of electrons through the ATPase enzyme, generating electrochemical force for the synthesis of ATP, coupling the corrosion reactions with respiration bacteria of Fe (III) (Dawood & Brozel 1998).

Numerous environmental factors influence the corrosion capacity of microorganisms, including *Bacillus* species. For example, temperature gradients are commonly reported in studies on biocorrosion in marine environments, usually associated with oil pipelines (Conrad et al. 2009; Yang et al. 2016; Li et al. 2017). Differences in environmental pH also

determine the speed of the MIC, directly affecting the adhesion between microorganisms and the metal surfaces (Sheng et al. 2008). In addition, interactions between different species present in the corrosive biofilm determine synergism or antagonism during the corrosion process (Suma et al. 2019; Kokilaramani et al. 2020). The concentration of NaCl and nitrate has also been reported as factors that influence MIC. In fact, a study shows that the concentration of NaCl affects the maintenance of biofilm structures (Dong et al. 2011). Although nitrate is directly associated with increased corrosion by nitrogen-reducing bacteria (NRB), in a study on the addition of nitrate and urea, corrosion by aerobic bacteria was inhibited (Pillay and Lin 2013 and 2014; Lin and Madida 2014). The involvement of heterotrophic bacteria of the genus *Bacillus* is widely described in marine corrosion studies, usually present in complex structures of microbial communities adhered to alloy surfaces (Selvaraj et al. 2014). *B. cereus* is a Gram-positive rod-forming bacteria that can grow in the presence or absence of oxygen and is widely distributed in many biotopes and in many different environments. The present work describes the *B. cereus* Mc-1 strain isolated from the marine environment close to the ship's maintenance area. DNA sequencing techniques were used to identify the isolate. Efforts were made to characterize the formation of biofilm in different cultivation conditions, to verify the most significant role in the induction/acceleration of corrosive processes in 1020 carbon steel, 316L stainless steel and copper coupons.

Materials and methods

Preparation of the specimens, bacteria, and culture medium

The *B. cereus* Mc-1 bacteria isolated in a previous study was grown in a nutrient broth medium up to absorbance of 1.0 at a wavelength of 600 nm. After satisfactory growth, the culture was centrifuged at 8,000 g, then the supernatant was discarded and the precipitate was washed with Phosphate Buffered Saline (PBS) (Merck, Germany). The centrifugation and washing processes were repeated twice. Then, the cell pellet was used for inoculation in 3 ml of broth medium for growth of iron precipitating bacteria ammonia ferric citrate medium (CFA), which was designed based on the chemical composition of 0.5 g/l $(\text{NH}_4)_2\text{SO}_4$, 0.2 g/l $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l NO_3^- , and 10.0 g/l ammonia ferric citrate, 0.5 g/l K_2HPO_4 , and the pH adjusted for 6.6 using 6 N HCl. After 48 h the culture was centrifuged, the supernatant discarded and the precipitate was used to inoculate for the corrosion experiment (described below).

Coupon preparation and experimental setup

Three different metal alloys were employed. ASI-1020 carbon steel, which has a chemical composition of C = 16%, Mn = 0.63%, P = 1.2%, S = 3.1%, Si = 1.2%, Cu = 1%, Cr = 3% and Ni = 1%; 316L stainless steel with maximum chemical composition of 0.030% C, 2.00% Mn, 0.75% Si, 0.045% P, 0.030% S, from 16.00 to 18.00% Cr, from 10.00 to 14.00% Ni, from 2.00% to 3.00% Mo, and copper Cu = 99.9%, Sb = 0.002%, Bi = 0.001%, As = 0.002%, Fe = 0.005%, Pb = 0.005%, and S = 0.005%. The carbon steel coupons were cut to 3 cm², whereas the 301L stainless steel and copper coupons were cut to 4 cm². They were exposed to absolute ethanol to degrease the surface, washed with acetone to remove organic matter, dried in an oven 70 °C for 30 min, and kept in a desiccator. After the coupons were sterilized by autoclaving and then were cooled, identified, and weighed, to evaluate the weight loss by corrosion.

The determination of corrosion was done using the treated coupons placed in the bottom of the Erlenmeyer glass of 250 ml, and the growth medium was the CFA medium without the ammonia ferric citrate compound (CFA.Ico-). Initially, a pre-inoculum was prepared from an isolated colony of *B. cereus* Mc-1 strain in CFA medium for 72 h under the same conditions described above. After the growth reached the optical density of 0.50 at a wavelength of 600 nm, 100 µl were withdrawn and used to inoculate the CFA.Ico- medium with the coupons. Were analyzed the mass loss by microbial corrosion after 7 and 15 days of exposition to CFA.Ico-. All analysis was performed in triplicate. To analyze the influence of other nutrients in the corrosion rate by Mc-1 strain, additional coupons assays were performed followed by the same parameters described above with supplements of the individual compounds: 2.0 gL⁻¹ NaCl and 2.0 gL⁻¹ of NO₃-.

Weight loss determination

After each determined period for analysis, three coupons from each system were retrieved for the measure of mass loss and corrosion rate. The rust deposited was scraped, and the coupons were immediately immersed in acid pickling (15% HCl) to remove all surface corrosion products according to ASTM G1 (Standard Practice for Preparing, Cleaning, and Evaluating Corrosion Test Specimens) (ASTM G1-03 2017). The acid reaction was stopped by the application of thiourea solution for 5 s, next the coupons were washed with distilled water, and then the reaction was neutralized with 10% NaOH for 5 s, and finally immersed in acetone for the same period. To determine the corrosion rate during the experimental period of incubation in all conditions, the weight-loss evaluation method was employed. The results of weight-loss measurement were utilized to calculate the value of corrosion rates (CR) following the equation:

$$CR = (W \times 365 \times 1000) / (D \times A \times T),$$

where the *W* is the decrease in metal weight during the time period analyzed, *K* is the constant (3650), *D* the metal density in g/cm³, *A* the coupon area (mm²), and *T* the exposure time in days (NACE 2005). To evaluate the significant differences in the corrosion rates, an analytical analysis *t* test was employed.

Results

The strain *B. cereus* Mc-1 was previously isolated in a screening experiment to oxidize carbon steel bacteria. In the present study, the Mc-1 strain was analyzed if, under conditions of presence of NaCl and NO₃, it favored its corrosive metabolism. Initially, *B. cereus* Ms-1 was grown in the CFA.Ico- medium in the presence of carbon steel coupons, for a period of up to 30 days. In the presence of the Mc-1 strain, weight loss was greater than in the control system, being 0.126 mg (±0.03) after 7 days, 0.382 (±0.13) in 15 days, and 0.517 (±0.07) in 30 days cultivation (Fig. 1a and Table 1). The values of corrosion rates were also considerably higher, 279.58 (±83.5) in 7 days, 394.61 (±137.4) after 15 days, and 534.2 (±82.2) after 30 days (Fig. 1a and Table 1).

When NaCl was added to the culture medium there was a decrease in the values of weight loss, 0.54 mg (±0.01) in 7 days, 0.20 (±0.05) in 15 days, and 0.26 (±0.01) in 30 days of exposition, and in corrosion rates, 120.22 (±40.8), 209.27 (±59.7), and 275.08 (±19.5) in the same period (Fig. 1b and Table 1). The addition of NO₃- to the culture media showed an acceleration of mass losses, with values of 0.15 mg (±0.01), 0.35 mg (±0.04), 0.39 mg (±0.08) in the three periods analyzed, and corrosion rates, 343.19 (±32.6), 369.71 (±42.6), and 402.83 (±88.1) in the same incubation periods, concerning the previous experiment, as well as the control. (Fig. 1c and Table 1).

Corrosion rates influenced by the *B. cereus* Mc-1 strain were also detected in 316L stainless steel coupons. In the CFA.Ico- medium, the weight loss results showed that there was a moderate increase in levels over the experimental period, with 0.006 mg (±0.001) in 7 days, 0.011 mg (±0.009) in 15 and 0.024 mg (±0.003) after 30 days (Table 1). In corrosion rates, a similar pattern was described, with 0.011 (±0.009) in 7 days, reaching 18.83 (±2.8) after 30 days of growth (Fig. 2a and Table 1). The addition of NaCl to the culture medium did not show significant changes between the periods analyzed. Weight loss was 0.002 mg (±0.001) in 7 days, followed by 0.007 mg ±0.001 and 0.009 mg (±0.002) after 15 and 30 days, respectively. The corrosion rates also showed little change, with values of 4.12 (±3.2), 5.29 (±0.4), and 7.3 (±1.7) in the same periods analyzed (Fig. 2b and Table 1). The

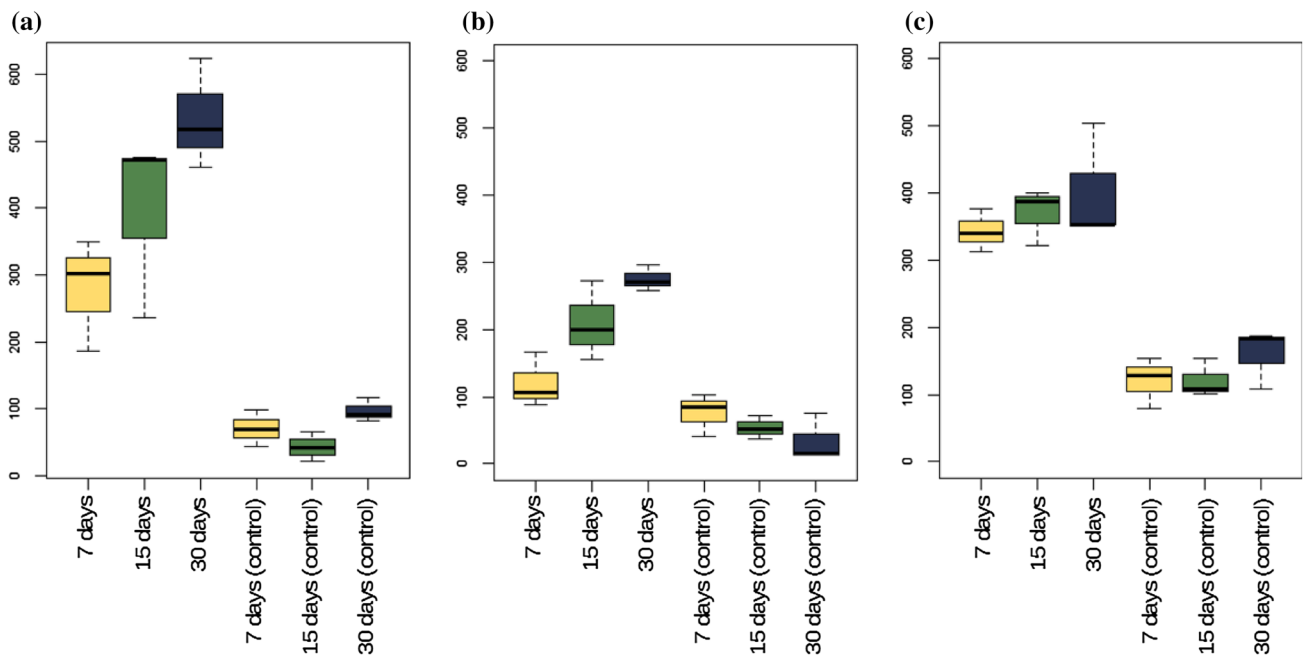


Fig. 1 Corrosion rates of carbon steel coupons after 7, 15 days, and 30 days. **a** CFA.ico- culture medium, **b** CFA.ico- culture medium with NaCl (2%) supplementation, and **c** CFA.ico- culture medium with NO_3^- (2%) supplementation

analyzes after NO_3^- supplementation showed measures similar to those described in the previous condition, with weight loss values between 0.005 mg (± 0.001) and 0.009 mg (± 0.001), and a corrosion rate of 8.53 (± 1.4) in 7 days, 7.07 (± 1.5) in 15 days, and 6.79 (± 1.4) in 30 days (Fig. 2c and Table 1).

The influence of *B. cereus* Mc-1 on the corrosion of copper coupons was also evaluated in this study. Under culture conditions in CFA.Ico medium, the loss of mass was constant throughout the experimental period, from 0.002 mg (± 0.001) in 7 days to 0.051 mg (± 0.008) after 30 days. The loss of mass was reflected in the corrosion rate, which was 2.9 (± 1.4) in 7 days, 8.6 (± 3.8) in 15 days, and 17.55 (± 2.8) after 30 days (Fig. 3a and Table 1). With the addition of NaCl to the culture medium, variations in the corrosion rate were negligible, e.g. 5.33 mg (± 0.83), 7.24 mg (± 2.57), and 8.82 mg (± 0.9) throughout the experimental period (Fig. 3b and Table 1). Finally, in the cultivation with NO_3^- added, the values of mass loss rose from 0.002 mg (± 0.001) in 7 days to 0.053 mg (± 0.001) after 30 days, whereas the corrosion rates varied from 4.31 (± 3.56) to 7 days, 14.1 (± 0.6) in 15 days, and 18.21 (± 2.5) after 30 days of culture (Fig. 3c and Table 1).

Discussion

B. cereus is a typical heterotrophic and aerobic bacterium widely distributed in the environment, such as soil, fresh water, and marine environments (Capão et al. 2020). The

Mc-1 strain was initially isolated from seawater in a previous study on the microbial diversity involved in the corrosion of carbon steel (Ribeiro et al. 2017). In this previous work, the Mc-1 strain was shown to be able to oxidize carbon steel at high levels and in a short period. In this study, the Mc-1 strain was evaluated for its ability to induce/accelerate the corrosion of two different metals, which are commonly used in offshore structures (Marconnet et al. 2008). Laboratory studies simulating corrosion processes influenced by microorganisms are a challenge. Although microcosms simulate the conditions found in the environment, several factors inherent to the corrosive process are difficult to be analyzed. However, controlled conditions in laboratories allow us to separately assess the main factors that influence corrosion of metals (Angell et al. 1997; Mumford et al. 2016). Other studies have already reported the corrosive capacity of *Bacillus* species in different laboratory conditions (Guo et al. 2017; Wan et al. 2018; Yuan and Pehkonen 2007; Li et al. 2019; Abdoli et al. 2016). The presence of *Bacillus* species has been reported in previous studies of corrosion in marine environments (Bonifay et al. 2017; Moura et al. 2018; Procópio 2020a). In metagenomic approaches and culture of isolates grown on the surfaces of oil pipelines and immersed coupons in marine environments the *Bacillus* genus was widely detected (Rajasekar et al. 2010; Garcia and Procópio 2020). In a study on the biofilm of *B. cereus* in carbon steel immersed in seawater, this species demonstrated to adhere to the surface under aerobic conditions (Procópio 2020c). However, the results on corrosion rates indicated that the

Table 1 Measures of weight loss and corrosion rate (C.R.) influenced by *B. cereus* Mc-1 strain on different metal surfaces and growth conditions

Metal	Medium	Period	Experimental system		Negative control	
			Weight loss	C.R	Weight loss	C.R
Stell carbon	CFA.Ico-	7 days	0.126 (\pm 0.03)	279.58 (\pm 83.5)	0.032 (\pm 0.01)	70.76 (\pm 27.1)
		15 days	0.382 (\pm 0.13)	394.61 (\pm 137.4)	0.04 (\pm 0.02)	42.61 (\pm 22.1)
		30 days	0.517 (\pm 0.07)	534.2 (\pm 82.2)	0.09 (\pm 0.01)	97.34 (\pm 18.1)
	CFA.Ico- + NaCl 2%	7 days	0.54 (\pm 0.01)	120.22 (\pm 40.8)	0.03 (\pm 0.01)	76.36 (\pm 31.8)
		15 days	0.20 (\pm 0.05)	209.27 (\pm 59.7)	0.05 (\pm 0.01)	53.62 (\pm 17.4)
		30 days	0.26 (\pm 0.01)	275.08 (\pm 19.5)	0.05 (\pm 0.03)	37.8 (\pm 25.4)
	CFA.Ico- + NO ₃ 2%	7 days	0.15 (\pm 0.01)	343.19 (\pm 32.6)	0.05 (\pm 0.01)	120.59 (\pm 37.4)
		15 days	0.35 (\pm 0.04)	369.71 (\pm 42.6)	0.11 (\pm 0.02)	120.8 (\pm 28.7)
		30 days	0.39 (\pm 0.08)	402.83 (\pm 88.1)	0.015 (\pm 0.04)	159.43 (\pm 43.7)
Stainless 316L	CFA.Ico-	7 days	0.006 (\pm 0.001)	11.14 (\pm 2.5)	0.003 (\pm 0.001)	5.48 (\pm 1.4)
		15 days	0.011 (\pm 0.009)	8.51 (\pm 7.3)	0.004 (\pm 0.002)	3.54 (\pm 1.8)
		30 days	0.024 (\pm 0.003)	18.83 (\pm 2.8)	0.009 (\pm 0.001)	6.31 (\pm 0.8)
	CFA.Ico- + NaCl 2%	7 days	0.002 (\pm 0.001)	4.12 (\pm 3.2)	0.004 (\pm 0.001)	5.81 (\pm 0.7)
		15 days	0.007 (\pm 0.001)	5.29 (\pm 0.4)	0.005 (\pm 0.002)	3.87 (\pm 1.4)
		30 days	0.009 (\pm 0.002)	7.3 (\pm 1.7)	0.007 (\pm 0.004)	5.8 (\pm 3.3)
	CFA.Ico- + NO ₃ 2%	7 days	0.005 (\pm 0.001)	8.53 (\pm 1.4)	0.002 (\pm 0.001)	3.91 (\pm 0.4)
		15 days	0.009 (\pm 0.001)	7.07 (\pm 1.5)	0.005 (\pm 0.001)	3.75 (\pm 0.9)
		30 days	0.009 (\pm 0.001)	6.79 (\pm 1.4)	0.004 (\pm 0.001)	3.09 (\pm 0.9)
Copper	CFA.Ico-	7 days	0.002 (\pm 0.001)	2.9 (\pm 1.4)	0.001 (\pm 0.001)	1.45 (\pm 0.18)
		15 days	0.012 (\pm 0.005)	8.6 (\pm 3.8)	0.001 (\pm 0.001)	0.95 (\pm 0.3)
		30 days	0.051 (\pm 0.008)	17.55 (\pm 2.8)	0.008 (\pm 0.003)	2.85 (\pm 0.8)
	CFA.Ico- + NaCl 2%	7 days	0.003 (\pm 0.001)	5.33 (\pm 0.83)	0.002 (\pm 0.001)	6.42 (\pm 0.8)
		15 days	0.010 (\pm 0.003)	7.24 (\pm 2.57)	0.005 (\pm 0.002)	4.62 (\pm 0.4)
		30 days	0.026 (\pm 0.002)	8.82 (\pm 0.9)	0.012 (\pm 0.002)	6.18 (\pm 0.8)
	CFA.Ico- + NO ₃ 2%	7 days	0.002 (\pm 0.001)	4.31 (\pm 3.56)	0.003 (\pm 0.001)	5.87 (\pm 1.6)
		15 days	0.021 (\pm 0.003)	14.1 (\pm 0.6)	0.005 (\pm 0.001)	4.85 (\pm 0.1)
		30 days	0.053 (\pm 0.001)	18.21 (\pm 2.5)	0.014 (\pm 0.002)	4.15 (\pm 0.7)

B. cereus biofilm played an inhibiting role in the corrosion of carbon steel (Aïmeur et al. 2015). In addition, another study on the corrosive action of *Bacillus subtilis* alongside *Pseudoalteromonas lipolytica* showed that *B. subtilis* had an inhibitory action on *P. lipolytica*-induced corrosion (Guo et al. 2017). The presence of other electron acceptors influences the corrosion rate of metals, including the presence of nitrate (Kato et al. 2015; Wan et al. 2018). An evaluation of the addition of nitrate to the culture medium showed that *Bacillus licheniformis* consistently induced pitting corrosion in C1018 carbon steel (Xu et al. 2013).

Corrosion reduction results were also obtained with the *B. subtilis* C2 strain after a period of initial corrosion (Wang et al. 2020). Other studies conducted with the genera *Bacillus* and *Pseudomonas* showed similar results (Xu et al. 2016; Li et al. 2019). The electrochemical analysis showed that *Pseudomonas aeruginosa* significantly reduced corrosion in duplex stainless steel (DSS) (Xu et al. 2016). Furthermore, a study on *B. cereus* grown in synthetic seawater showed that the presence of the biofilm had a protective action in

the formation of pitting in the non-oxidizing action 316L (Li et al. 2019; Capão et al. 2020). Colonization of copper alloy surfaces by species of the *Bacillus* genus is common in marine environments under aerobic conditions (Guo et al. 2017; Zhang et al. 2019). Despite that the corrosive action of species of the genus *Bacillus* has been previously reported on copper surfaces (Hu et al. 2019), historically this genus has been described as an inhibitory role in the corrosion of these surfaces (Jayaraman et al. 1999; Guo et al. 2017; Moradi et al. 2019).

Conclusions

The bacterium *B. cereus* Mc-1 showed different corrosive behavior under different culture conditions and different metals evaluated. By analyzing the influence on corrosion of 1020 carbon steel and copper alloy, Mc-1 demonstrated to accelerate the corrosion process in the CFA.ico- and CFA.ico medium with NO₃ supplementation. On the other

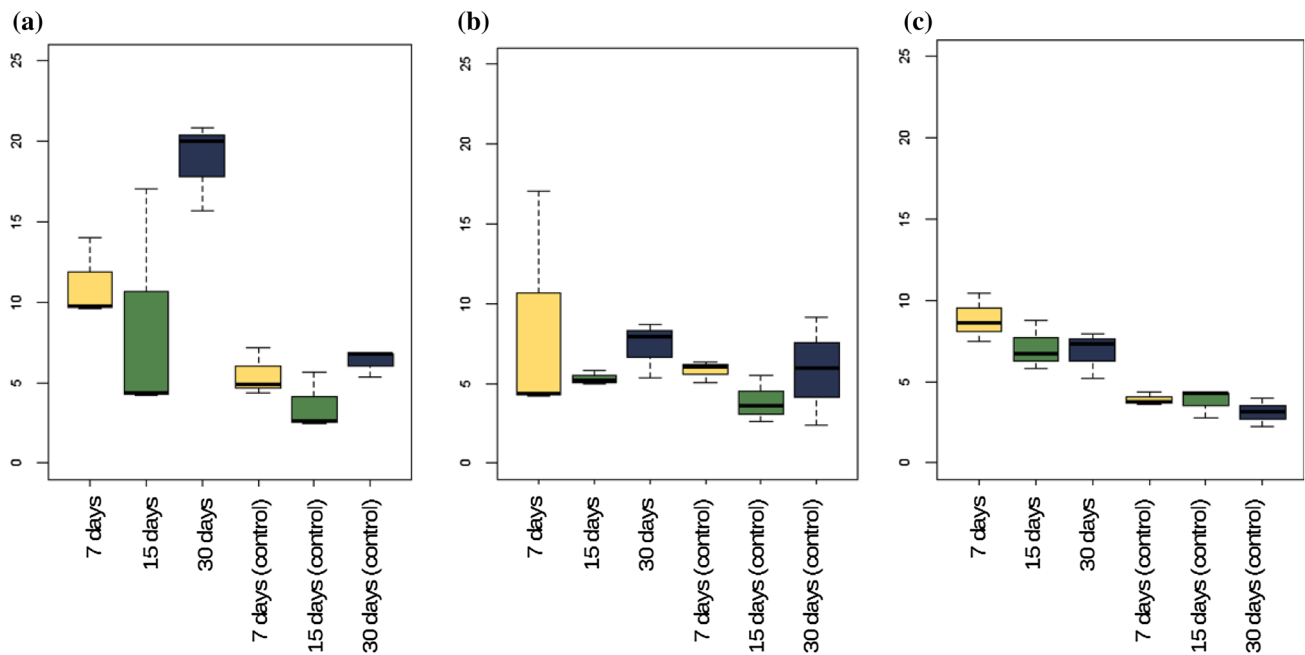


Fig. 2 Corrosion rates of 316L stainless steel coupons after 7, 15 days, and 30 days. **a** CFA.ico- culture medium, **b** CFA.ico- culture medium with NaCl (2%) supplementation, and **c** CFA.ico- culture medium with NO_3^- (2%) supplementation

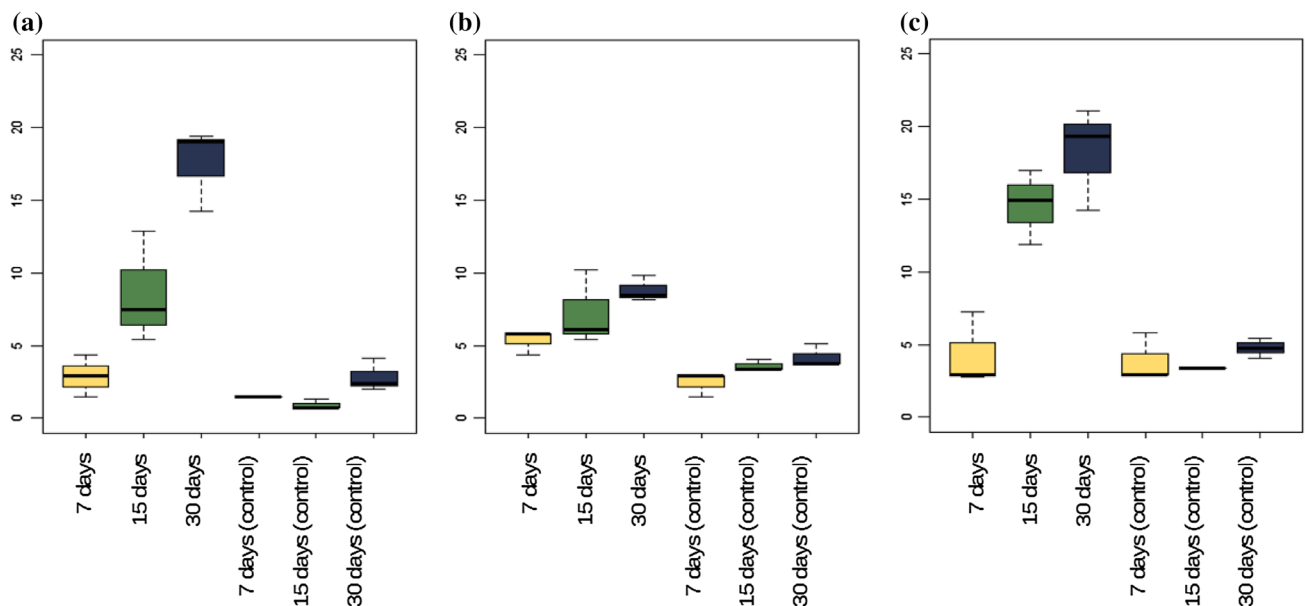


Fig. 3 Corrosion rates of copper alloys coupons after 7, 15 days, and 30 days. **a** CFA.ico- culture medium, **b** CFA.ico- culture medium with NaCl (2%) supplementation, and **c** CFA.ico- culture medium with NO_3^- (2%) supplementation

hand, the addition of NaCl caused a deceleration of corrosion in carbon steel. When analyzing the measurements of the 316L coupons, the results showed that after a period of corrosion induced by Mc-1, there was a persistent decrease in metal corrosion in all cultivation conditions analyzed.

These results bring new subsidies for the understanding of the biocorrosion by the bacterium *B. cereus* Mc-1 in different metallic surfaces and environmental conditions. These possible findings assist in mitigating biocorrosion influenced by microorganisms.

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