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Steel corrosion by iron oxidant bacteria isolated from sea water

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Abstract

The metal corrosion is a spontaneous process accountable for numerous problems for the industry. The corrosion of metals can be classified into four different types, among which is the microbiologically influenced corrosion (MIC). Among the groups of bacteria responsible for biocorrosion process threre are the Iron oxidizing bacteria (FeOB), which acquire energy required for their metabolism by iron oxidation mechanism, causing great damage to equipment and metal structures. Were isolated using techniques cultures in appropriate media oxidizing iron bacterial colonies form sea water samples near from vessels maintenance areas. To assess the oxidative capacity of the biofilm each colony was carried out the corrosion test of carbon steel coupons AISI-1020, the end of the experiment using the corrosion rate calculation for weight loss. The two colonies that showed a higher rate and corrosion were subjected to sequencing their 16S ribosomal genes and phylogenetic analysis. The identification results showed that the colony M14 has homology with *Pseudomonas stutzeri* and the colony M28 homology with *Bacillus cereus*. This study showed that colonization of bacteria on metal surfaces can accelerate abruptly corrosion of metallic alloys used in industry.

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Introduction

Metal corrosion is a spontaneous process that causes numerous problems for the industrial sector, causing enormous economic losses that reach about US$2.5 trillion, equivalent to roughly 3.4% of the global Gross Domestic Product (GDP) (NACE, 2016). Corrosion of metals can be classified into four different types, although this division is not restricted: (1) generalized corrosion, with little localized penetration on the metal; (2) pitting, characterized as localized and deep penetration into the metal; (3) galvanic corrosion, which generally occurs when metals of different potential redox are in contact, forcing the metal with greater redox potential to become anode and accelerate the rate of corrosion and (4) microbiologically influenced corrosion (MIC) or biocorrosion, which Is defined as corrosion initiated or aggravated due to the direct or indirect activity of microorganisms (Gentil, 2014).

The CIM presents the similar electrochemical nature than the traditional corrosions, which promote the degradation of metal structures exposed to the environment, in contact with oxygen, submerged in the soil, subsoil and in aqueous environments. The simultaneous occurrence of these factors forms an aqueous solution, called electrolyte, capable of conducting electricity, thus initiating or influencing the corrosion process on the metal (Little & Lee, 2014). Redox reactions occur simultaneously in aerated solutions, where the degradation of the metal occurs by gradient of oxygen concentration, in cathodic and anodic regions. The cathodic reaction, located under the drop of water, the metal undergoes dissolution by oxidation causing the release of metal ions in the solution, and the cathodic reaction occurs where the oxygen is reduced. The flow of electrons follows from the cathodic region to the anodic generating chemical change of the fundamental state of the metal to an ionized species (Beech & Gaylarde, 1999; Videla, 2003; Beech *et al.*, 2005).

The colonization of bacteria on metal substrates can directly influence oxidation reactions, affecting the anode/cathodic reaction kinetics. This biodegradation by participation of microorganisms has as more significant characteristic the modification of the interface metal/solution by the action of biofilms growth over the metal. Biofilms are dynamic and complex structures with different transport processes and chemical reactions that can induce important changes such as oxygen levels, pH and ion concentrations of micro environmental on the metal surface. The mechanism employed by microorganisms, through biofilm structures, occurs through the generation of conditions that increase or influence corrosion, as the oxygen concentration gradient by the development of microenvironments during biofilm growth, the excretion of corrosive metabolites such as enzymes, exopolymers, organic and inorganic acids that may affect the dynamics of redox reactions at the biofilm/metal interface (Dang *et al.*, 2011; Carpen *et al.*, 2013).

The main microorganisms described as related to the biocorrosion process are sulfate reducing bacteria (BRS) and thiosulfate-reducing bacteria (TRB) in anaerobic systems (Boudaud *et al.*, 2010). More recently, attentions have been directed to corrosive corrosion processes, especially metal corrosion, by presence of lithotrophic Fe-oxidizing bacteria (FeOB) and heterothrophic bacteria, as *Bacillus* and *Pseudomonas* strains (Chongdar *et al.*, 2005; McBeth & Emerson, 2016; Marty *et al.*, 2014; Lee & Newman, 2003). The Fe-oxidizing bacteria are autotrophic with great structural diversity, but present the same principle of obtaining energy, the ability of oxygen to oxidize ferrous ion (Fe2 +) to ferric ion (Fe3 +) with subsequent formation of insoluble precipitates of oxides ($Fe_2O_3.H_2O$) or iron hydroxides ($Fe(OH)_3$) as brownish or orange tubers in the metal substrates. The biocorrosion caused by this type of bacteria is frequently induced by a specific heterogenic group, the genera *Gallionella*, *Sphaerotillus*, *Crenothrix* and *Leptothrix* (Lee & Newman, 2003; Vidella, 2003; Hedrich *et al.*, 2011).

Sea water is considered an extremely corrosive medium of high complexity due to its chemical nature and biological characteristics favorable to the development of a wide variety of microbial species. Submerged biotic and abiotic surfaces in marine

environments are rapidly colonized by microorganisms. The colonization of these surfaces and the subsequent formation and development of the biofilm promotes innumerable advantages to these microorganisms as it supports critical changes in the ecological and biogeochemical functions of the environment (Araujo *et al.*, 2013, Gentil, 2014; Dang & Lovell, 2015).

In the present work we performed a screening for marine bacteria involved in corrosive processes over metal coupons of carbon steels. The major bacteria evaluated as influencers in the corrosion processes were isolated, identified as by DNA sequencing, and evaluated for their corrosion kinetics.

Material and methods

Sample collection

The samples collection were conducted using seawater collected near the maintenance area ships, in Rio de Janeiro city, RJ-Brazil, in sterile 2 liter-bottles, and carried immediately to laboratory. The isolation of bacteria was carried out by the serial dilution of collected water and spread over Marine Broth medium (Difco, USA) with previously added agar. The plates were incubated in dark, at 18°C, up to 72 hours in aerobic conditions. Isolate colonies were picked up and transferred for marine and nutrient agar slants for short term storage. After satisfactory growth the colonies in slants, the isolates were 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 0.5g/l $(NH_4)_2SO_4$, 0.2g/l $CaCl_2.6\ H_2O$, 0.5g/l $MgSO_4.7\ H_2O$, 0.5g/l $NaNO_3$ and 10.0g/l Ammonia Ferric Citrate, 0.5g/l K_2HPO_4, the pH was adjusted for 6.6 using HCl 6N, and were incubated at same conditions described above. The positive iron precipitating bacteria were visually recognized by the change of color of the medium, from green to red-rust, indicating the oxidation of iron present in broth medium. Colonies with growth satisfactory in Marine Broth medium with agar were analyzed in CFA medium after 10 and 15 days, and positive iron precipitating were separated and used for steel coupon corrosion experiment.

Corrosion experimental set up

AISI-1020 steel coupons used in all experimental systems had measures about 1.0 cm^2 and chemical composition C=16%, Mn=63%, P=1.2%, S = 3.1%, Si=1.2%, Cu=1%, Cr=3% and Ni=1%. The coupons were progressively exposed to silica microsphere jets, for complete cleaning the cover material, following for incubation with absolute ethanol to degrease the surface, washed with acetone to remove organic matter, dried in an oven 70°C for 30 minutes and kept in a desiccator. After the coupons were sterilized by autoclaving and then were cooled, identified and weighed, in order to evaluate the weight loss by corrosion.

Determination of corrosion was done using the treated coupons placed in bottom of Erlenmeyer glass of 250ml, and the growth medium was the CFA medium without the Ammonia Ferric Citrate compound (CFA.Ico-). The positive iron precipitating colonies were inoculated in CFA.Ico-medium with the coupons. Were analyzed the growth bacteria and mass loss by microbial corrosion after 30 days of exposition to CFA.Ico-medium. All analysis were performed in triplicate.

Weight loss determination

Evaluation of corrosion progress was done using the weight loss method as described by Rabald Index (Rabald, 1968). To determine the weight loss on carbon steel coupons was adopted and NACE Standard RP 0775 (NACE, 2005), which establishes the levels of corrosivity on carbon steel by the loss weight. The weight loss (dw) was calculated by the following equation:

dw = S.10/d (g/cm3)

where S is the exposed surface area of steel coupon, mm 2 and d is metal density. From the results of weight loss measurement, the value of corrosion rates (v) could be calculated by the following equation:

v = dw . 365/t

where t is the exposure time in days.

At each sampling timepoint, three coupons were removed and one milliliter of supernatant.

The covered biofilm were scraped, and the biofilm scraped and the supernatant were used for biofilm assay described below. The coupons were immediately immersed in 26% hydrochloric acid, inhibited with thiourea solution for 5 seconds, washed with distilled water, then neutralized with 10% NaOH for 5 seconds and washed again with sterile distilled water. After, they were immersed in absolute ethanol for 5 seconds followed by immersion in acetone at the same period. The coupons were dried in a desiccator for 20 minutes until they reached constant weight, the final the corrosion rate determined by for weight loss.

Identification

Representative colonies were growth in nutrient broth medium up to absorbance of 1.0 at wavelength of 600nm. Then, an aliquot of 1ml was placed in 1,5mL sterile tube and sent for Neoprospecta Microbiome Technologies Technologies (Florianópolis-SC, Brazil). The sample preparation and sequencing was performed by Neoprospecta Microbiome Technologies Technologies. It consist in the 16S rRNA V3/V4 region was amplified using the 341F (5´-CCTACGGGRSGCAGCAG-3´) and 806R (5´-GGACTACHVGGGTWTCTAAT-3´) primers, with Illumina adapters, necessary for sequencing. The amplification was performed in 35 cycles at 50°C of annealing temperature, where, each sample was amplified in triplicate. The sequencing was performed in Illumina MiSeq, using V2 kit, with a single-end 300 nt run.

The bioinformatics analyses were performed by Neoprospecta Microbiome. The primers and adapters sequences were trimmed from the reads, and only sequences with 275nt or more were used in downstream analysis.

Then, all reads with one or more indeterminate bases "N" and truncated sequences with two or more consecutive bases with quality scores below to Q20, were eliminated. OTU Picking was performed using BLASTN 2.2.28 against GreenGenes 13.8 database. To attribute taxonomy, only sequences with hits of 99% of identity in an alignment covered over 99% were considered.

Results and discussion

Initially about 500 colonies previously growth in Marine medium were analyzed in CFA broth. After 10 and 15 days, about 23 colonies showed growth and iron precipitating in CFA medium. The positives growth could be visually recognized by the change of color of the medium as show in Fig. 1 (a) and (b). Microbial iron oxidation under mesophilic conditions it was always a challenge, mainly on solid media, maybe due to a number of factors as nutrient deficiency and complexing elements. The change in the color of the culture medium is caused by the growth of iron oxidizing bacteria whose metabolic activity causes the oxidation of the ferric ion present in the culture medium, generating insoluble precipitates of ferruginous iron oxide (Torres, 2011). Iron precipitating bacteria play an important role in metal corrosion processes by producing rust of oxides or iron hydroxide on the affected surface, which causes various mechanical damages in the metal structure (Gentil, 2011). In addition, these precipitates facilitate the subsequent growth of anaerobic bacteria, by hindering the diffusion of oxygen to the metallic substrate, such as sulfate-resisting bacteria, which are widely described in the literature for several corrosive damages on metal surfaces (Boudaud *et al*., 2010).

Fig. 1. (A) and (B) show the growth of iron precipitating bacteria in CFA broth medium after 10 and 15 days, respectively.

Twenty-three colonies were evaluated for their ability to induce or accelerate the corrosion of 1020 carbon steel coupons under aerobic conditions. The bacteria were incubated under agitation in Erlenmeyer flasks for a period of 30 days (Fig. 2). During the experiment of bacteria growth on the coupons, a small amount of brow deposit could be seen on the surfaces of steel coupons after few days and, throughout the experiment, the layer of deposit became darker and thicker (Fig. 3). The formation of rust deposits associated with the formation of bacterial biofilm structures on carbon steel surfaces is commonly documented in the scientific literature. In aqueous environments, such as marine environments, the adsorption of bacteria on surfaces is frequent, forming exopolymeric streaks from the secretion of products synthesized by colonizing bacteria of solid surfaces. Once these biofilm structures are formed, the microbial activity changes the physicochemical conditions, creating a micro environment conducive to the acceleration or inhibition of corrosion (Beech & Sunner, 2004). These complex structures harbor dense multicellular communities with different nutritional requirements, in which a communication between them is established through excreted materials, such as acidic substances and enzymes, during biofilm formation (Wood *et al.*, 2011; Videla & Herrera, 2005).

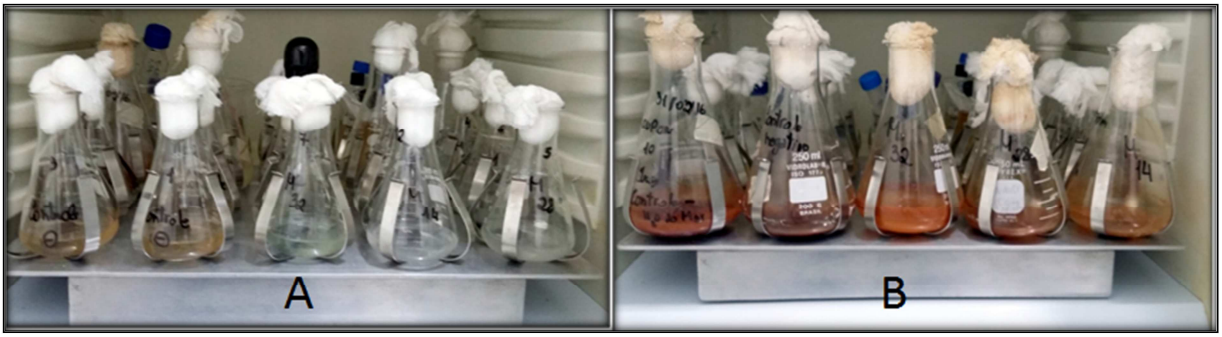

Fig. 2. Corrosion experiments in first day (A) and after 30 days (B), respectively.

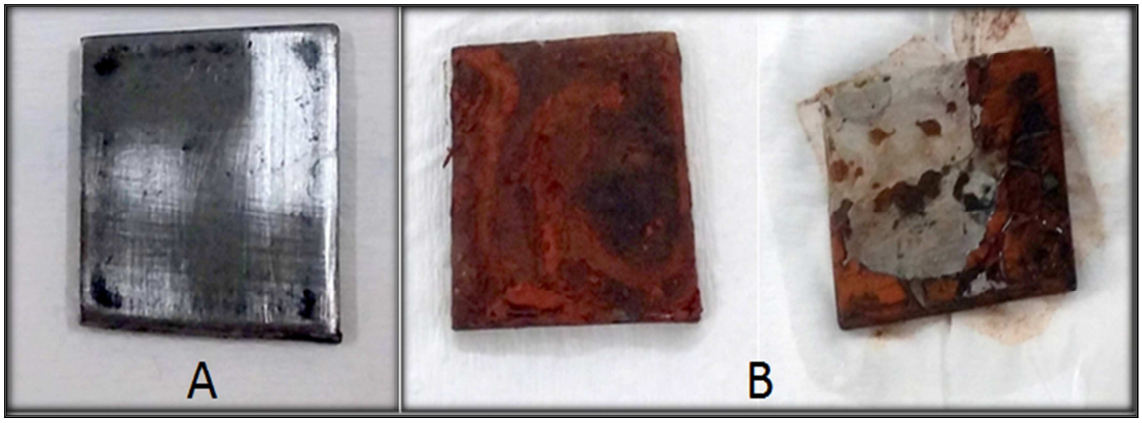

Fig. 3. Steel coupon prepared for corrosion experiments (A) and coupons after 30 days of corrosion experiments, respectively.

At the end of the experiment, three inoculums showed visually greater deposit of rust and associated bacterial growth. These isolates were separated and their corrosion kinetics evaluated after 30 days of incubation. At the end of the experiment, the coupons were prepared by the acid pickling process, and their mass loss corrosion rate of each AISI-1020 carbon steel coupon was calculated. In Table 1 the mean values of corrosion rate expressed in mm / year according to the Rabald index, the standard deviation of each mean and the corrosivity classification of the test bodies exposed to different media are presented. It was also verified that the values of the average corrosion rate of the exposed specimens to colony

M28, M32, M14 were respectively 57.6%, 40.8% and 45.5% higher than the average corrosion rate of the coupons Exposed to the control medium.

Table 1. Corrosion rates on steel coupons of isolated bacterial colonies selected.

Coupons	Average corrosion rate	Standard deviation	Corrosivity classification (NACE)
Control	0,42	±0,017	High
Colony M14	0,77	±0,011	Severe
Colony M28	0,99	±0,010	Severe
Colony M32	0,71	±0,036	Severe

The M14 and M28 isolates, that obtained the best results in the corrosion assay, were selected for identification through the sequencing of the 16S rRNA gene. Our sequencing analysis showed that the M14 colony has homology with the species *Pseudomonas stutzeri*, with a similarity index of 100%, whereas the colony M28 has homology with the *Bacillus cereus* species, with a similarity index of 100% also. In the work carried out on the role of microorganisms that cause biocorrosion in metal bodies that remained in direct contact with fresh water, several bacterial groups were identified: iron oxidants, sulfate reducing bacteria, aerobic bacteria and facultative anaerobic bacteria. Such as those belonging to the families Enterobacteriaceae and Vibrionaceae, to the genera *Bacillus* and *Pseudomonas*, as well as fungi (Mangaroni, 2010). The presence of species of the genus Pseudomonas is also described in several works of corrosion. The marine *Pseudomonas aeruginosa* strain showed able to initiate deep pits during corrosion process over 2205 and 2707 duplex stainless steels (Xu *et al.*, 2017; Li *et al.*, 2016). The role of both *Bacillus subtilis* and *Pseudoalteromonas lipolytica* species on corrosion also is described in recent study during biofilm formation over low-alloy steel plates (Guo *et al.*, 2017).

Conclusion

Our screening studies on the role of marine bacteria in the process of corrosion in steel alloy describe the presence of heterotrophic strains that act as colonizers and biofilm forming agents.

Our results describe a clear involvement in the initiation or participation of corrosive processes in sterile metal coupons. We believe that these bacterial species play a preponderant role in initial processes of metallic corrosion in aqueous systems, especially marine environments.

References

Araújo LCA. 2011. Avaliação da corrosão induzida microbiologicamente em aço carbono AISI 1020 revestido com tinta pigmentada com óxido de nióbio. Master Dissertation, Federal University of Rio de Janeiro p. 127.

Beech IB, Gaylarde CC. 1999. Recent advances in the study of biocorrosion: An overview. Reviews Microbiology **3(3),** 117-190.

Beech IB, Sunner JA. 2004. Biocorrosion: towards understanding interactions between biofilms and metals. Current Opinion in Biotechnology **15,** 181-186.

Boudaud N, Coton M, Coton E, Pineau S, Travert J, Amiel C. 2010. Biodiversity analysis by polyphasic study of marine bacteria associated with biocorrosion phenomena. Journal Applied Microbiology **109(1),** 166-179.

Carpén L, Rajala P, Vepsäläinen M, Bomberg M. 2013. Corrosion behaviour and biofilm formation on carbon steel and stainless steel in simulated repository environment. Eurocorr. poster 1589.

Chongdar S, Gunasekaran G, Kumar P. 2005. Corrosion inhibition of mild steel by aerobic biofilm. Electrochimica Acta **50,** 4655-4565.

Dang H, Chen R, Wang L, Shao S, Dai L, Ye Y, *et al.*, 2011. Molecular characterization of putative biocorroding microbiota with a novel niche detection of Epsilon- and Zetaproteobacteria in Pacific Ocean coastal seawaters. Environmental Microbiology **13(11),** 30593074.

Dang H, Lovell CR. 2015. Microbial surface colonization and biofilm development in Marine environments. Microbiology and Molecular Biology Reviews **80(1),** 91-138.

Gentil, V. 2011. Corrosão, LTC – Livros Técnicos e Científicos, Rio de Janeiro, p. 392.

Guo Z, Liu T, Cheng YF, Guo N, Yin Y. 2017. Adhesion of *Bacillus subtilis* and *Pseudoalteromonas lipolytica* to steel in a seawater environment and their effects on corrosion. Colloids Surfaces Biointerfaces **157,** 157-165.

Hedrich S, Schlömann M, Johnson DB. 2011. The iron-oxidizing proteobacteria. Microbiology **157(6),** 1551-1564.

Koch G, Varney J, Thompson N, Moghissi O, Gould M, Payer J. 2016. International mesasures of prevention, application, and economics of corrosion technologies study. NACE, Houston p. 216.

Lee AK, Newman DK. 2003. Microbial iron respiration: impacts on corrosion processes. Applied Microbiology Biotechnology **62(2-3),** 134-139.

Li H, Zhou E, Zhang D, Xu D, Xia J, Yang C, *et al.* 2016. Microbiologically Influenced Corrosion of 2707 Hyper-Duplex Stainless Steel by Marine *Pseudomonas aeruginosa* Biofilm. Science Reports **6,** 20190, p. 12.

Little BJ, Lee JS. 2015. Microbiologically influenced corrosion: an update. International Materials Reviews **56(7),** 384-393.

Marangoni PRD. 2010. Caracterização de biofilmes formados em superfícies metálicas e biocorrosão. Master Dissertation, Federal University of Paraná p. 103.

Marty F, Gueuné H, Malard E, Sánchez-Amaya JM, Sjögren L, Abbas B, *et al.* 2014. Identification of key factors in Accelerated Low Water Corrosion through experimental simulation of tidal conditions: influence of stimulated indigenous microbiota. Biofouling **30(3),** 281-297.

Mc Beth JM, Emerson D. 2016. In Situ Microbial Community Succession on Mild Steel in Estuarine and Marine Environments: Exploring the Role of Iron-Oxidizing Bacteria. Frontiers Microbiology **7,** 767.

NACE RP-07-75. 2005. Standard recommended practice, preparation, installation, analysis and interpretation of corrosion coupons in oilfield operations. NACE, Houston p. 19.

Rabald V. 1968. Corrosion Guide. Elsevier p. 265.

Torres ES. 2001. Cinética de parâmetros microbiológicos na formação de biofilmes. Master Dissertation. Rio de Janeiro p. 80.

Videla HA, Herrera LK. 2005. Microbiologically influenced corrosion: looking to the future. International Microbiology **8,** 169-180.

Videla HA, Herrera LK. 2009. Understanding microbial inhibition of corrosion. A comprehensive overview. International Biodeterioration and Biodegradation **63,** 896-900.

Videla HA. 2003. Biocorrosão, biofouling e biodeterioração de materiais. Editora Edgard Blücher LTDA, São Paulo p. 148.

Wood TK, Hong SH, MA Q. 2011. Engineering biofilm formation and dispersal. Trends in Biotechnology **29(2),** 87-94.

Xu D, Xia J, Zhou E, Zhang D, Li H, Yang C, *et al.* 2017. Accelerated corrosion of 2205 duplex stainless steel caused by marine aerobic *Pseudomonas aeruginosa* biofilm. Bioelectrochemistry **113,** 1-8.