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ORIGINAL PAPER

Corrosion behavior of carbon steel in the presence of two novel iron-oxidizing bacteria isolated from sewage treatment plants

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Abstract In this work, two novel iron oxidizing bacteria (IOB), namely *Gordonia* sp. MZ-89 and *Enterobacter* sp. M01101, were isolated from sewage treatment plants and identified by biochemical and molecular methods. Then, microbially influenced corrosion (MIC) of carbon steel in the presence of these bacteria was investigated. The electrochemical techniques such as potentiodynamic polarization measurements and electrochemical impedance spectroscopy (EIS) were used to measure the corrosion rate and observe the corrosion mechanism. The results showed that the existence of these microorganisms decreased the corrosion potential and enhanced the corrosion rate. Scanning electron microscopy (SEM) images revealed the ground boundary attacks and pitting on carbon steel samples in the presence of these bacteria after polarization. Corrosion scales were identified with X-ray diffraction (XRD). It was demonstrated that these bacteria can greatly affect the crystalline phase of corrosion products that also confirmed by SEM results. It was inferred that these bacteria were responsible for the corrosion of carbon steel, especially in the form of localized corrosion.

Keywords Microbially influenced corrosion · Electrochemical impedance spectroscopy · Iron oxidizing bacteria · Localized corrosion · Grain boundary attack

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Introduction

Microbially influenced corrosion (MIC) is a complex interaction between the microbial population, the environment and the metal substrate (Unas et al. 2005; Valcarce et al. 2006). It would create a serious industrial problem that affects diverse processes (Rao et al. 2005; Dubiel et al. 2002). It is estimated that about 20% of annual damage due to corrosion in metals is resulted from microbial activities, of which a significant part is due to aerobic corrosion influenced by iron-oxidizing bacteria (Xu et al. 2007).

A great number of researches have been performed on the MIC of cast iron and steels by aerobic and anaerobic bacteria (Lee and Newman 2003; Angell and White 1995; Rajasekar et al. 2007; Teng et al. 2008). A wide range of microorganisms, including

aerobic, anaerobic bacteria and fungi have been shown to be effective on the MIC of different metals and their alloys such as steel, copper and aluminum (Yuan et al. 2009).

In addition, various cases of corrosion damage which caused by iron-oxidizing bacteria (IOB) have been also observed and reported (Starosvetsky et al. 2001, 2008). These bacteria generate energy for growth by oxidation of ferrous ions to ferric and probably participate in rusty slime formation (Starosvetsky et al. 2001, 2008; Chen et al. 2007).

Like many other corrosion processes, MIC is essentially due to electrochemical reactions. In general, microbial colonization of metals would influence the rate and the mechanisms of electrochemical reactions (Olesen et al. 2000), therefore electrochemical techniques are certainly good methods to evaluate monitor and understand MIC processes (Fonseca et al. 1998).

Due to the importance of these bacteria in corrosion of metals, the present study was designed to achieve a better understanding of the efficacy of the aerobic IOB on the basis of corrosion process characteristics of carbon steel. In this work, two kind of IOB separated from sewage treatment plants, isolated, and identified by biochemical and molecular methods. Then, their corrosion process characteristics and mechanism were studied by potentiodynamic polarization measurements, electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and X-ray diffraction (XRD) techniques.

Materials and methods

Isolation of bacteria, microbial cultivation and incubation

The corrosion products were collected using sterile metal loops from inside of pipelines of sewage treatment plants. For isolation of IOB, 1 g of corrosion product was transferred to 250 ml of Erlenmeyer flask containing 50 ml of Winogradsky nutrient medium (Atlas 1993) (g/l): 0.5 K_2HPO_4, 0.5 $NaNO_3$, 0.2 $CaCl_2$, 0.5 $MgSO_4{\cdot}7H_2O$, 0.5 NH_4NO_3 and 6.0 ammonium iron citrate (pH = 4.8) under aerobic chamber. Before using this solution, it was autoclaved at 121°C for 15 min. The IOB cultures were grown through vigorous shaking for 72 h at room temperature (25 ± 1°C). As an outcome of bacterial activity, the color of culture medium shifted gradually with time from yellow to an intense orange, which is due to the formation of ferric compounds by these bacteria. Later, the grown cultures were subcultured on nutrient agar for selective isolation. For the bacteria, individual colonies were purified by repeated streaking on nutrient agar plates.

Identification

Morphological and biochemical characteristics of the isolated bacteria were studied according to the Bergey's Manual of Systematic Bacteriology (Seneath et al. 1986). The strains were characterized based on their morphological and biochemical characteristics. The main strains identification was confirmed according to the observation of 16S rRNA gene. Amplification of gene encoding for small subunit ribosomal RNA was performed using eubacterial 16S rDNA primers (forward primer 5′-GCACAAGCGGTGG AGCATGT-3′ and reverse primer 5′-CCCGGGAAC GTATTCACC-3′). Polymerase chain reaction (PCR) was performed with a 25 µl reaction mixture containing 2 µl (30 ng/µl) DNA as the template, each primer at a concentration of 1.5 µl (15 pmol), each deoxynucleoside triphosphate (dNTP) at a concentration of 0.5 µl (10 mM), as well as 0.5 µl of *Taq* polymerase and 10× buffer at a concentration of 2.5 µl and dH_2O added up to 25 µl. PCR was carried out with a Mastercycler Personal (Eppendorf, Germany) with the following program: initial denaturation at 94°C for 2 min; 30 cycles of denaturation (30 s at 94°C), annealing (30 s at 56°C), and extension (1 min at 72°C); followed by a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis of a 10 µl aliquot through a 1% (w/v) agarose gel, stained with ethidium bromide, and visualized by UV transillumination. After that, they were sent to sequencing. The size of the 16S rRNA fragment which has been sent for sequencing was ~500 bp. The similarity of the obtained sequences was examined by a Blast search at NCBI (National Center for Biological Information).

Material

In this work, the carbon steel (CK45) by the following composition: C ≤ 0.45, Si ≤ 0.4, and Mn ≤ 1.4, balance Fe was used for the investigation. Metal

specimens with the dimensions 2 cm × 2 cm × 0.5 cm were used in this study. To create working electrodes, an electrical contact to each steel sample was provided by a length of copper wire (the copper wires covered by the rubber) which connected to the back side of each specimen and then, mounted in an epoxy resin, such a way that only 4 cm^2 of specimen was in contact with aggressive solution. After that, the specimens were ground using silicon carbide metallurgical paper up to grit size of 1,000. Finally, they were rinsed in distilled water and sterilized in 70% ethanol for 2 h prior to use.

The Bushnell Hass medium (BH); consisting of (g/l), $MgSO4{\cdot}7H_2O$ 0.2, $CaCl_2$ 0.02, NH_4NO_3 1, K_2HPO_4 1, KH_2PO_4 1, $FeCl_3$ 0.05 at pH = 7 was used throughout this corrosion study because this medium has the minimum nutrient and so similar to natural environment. By this way, the affect of other parameters for corrosion attack would be omitted.

Electrochemical tests

All electrochemical tests were carried out in a corrosion cell with three-electrode arrangements consisting of a saturated Ag/AgCl electrode as reference, a platinum sheet as counter electrode and a carbon steel sample as working electrode. The measurements were conducted by using an AUTOLAB™ Potentiostat–Galvanostat (PGSTAT30). EIS measurements were made at the open circuit potential by using a 5 mV amplitude sinusoidal signal over the frequencies ranging from 10 kHz to 10 mHz and the obtained data were analyzed using Zview2 software. The Tafel polarization measurements were made just after EIS tests. The potential was swept from Eocp −250 to Eocp +250 mV with a scan rate of 5 mV s^{-1}. The carbon steel samples for electrochemical measurements were exposed to BH medium for different period times (1 and 2 weeks) and put in the incubator at 30°C through shaking with rate of 100 rpm for optimum bacteria growth. In fact, media Stagnant and low velocity waters appear to deprive the iron bacteria of required oxygen and reduce its growth but moderate velocities encourage their growth (Rao et al. 2005). In each case duplicate experiments were conducted and differences between two series were only within ± 1%. Whenever the differences were large, the data were confirmed by a third test. Also, standard deviation (SD) has been calculated for obtaining the precision of the average of electrochemical results such as i_{corr} and R_p. SD is a measurement of the amount of variation in a data set. The smaller the calculated SD shows the more precise of the measurements (Morgan 1993).

Surface analysis

After exposing for 1 month under freely corroding conditions in medium containing IOB, the carbon steel samples were examined for their surface characteristics and corrosion features by SEM after sputter coating with gold. To immobilize the bacterial cells in order to confirm the adhesion of bacteria, the samples were immersed for 4 h in a 2% glutaraldehyde solution. Then, to characterize the morphology of corrosion by the SEM, the coupons were dehydrated by using four ethanol solutions (15 min each):25, 50, 75 and 96% successively.

XRD, using a JEOL Model JDX-8030 was applied to scan the corrosion products between 10 and 85°—2θ (the angle twice of diffraction, peak position) with copper Kα radiation (Ni filter) at a rating of 40 kV, 20 mA. The carbon steel samples were used directly for XRD analysis to determine the nature of oxides layers and corrosion products on their surfaces.

Results

Identification of the isolates

The physiological and biochemical characteristics of strains IOB are shown in the Table 1. PCR amplification of bacterial DNA with universal 16S rRNA primers yielded a fragment of ∼500 bp. On the basis of the partial sequence analysis of the gene encoding 16S rRNA, the two strains of IOB were classified as a species of the genus *Enterobacter* and *Gordonia*. A pair wise alignment (BLASTn) analysis of 16S rRNA sequence of the isolates IOB also indicated that the DNA sequence had the highest identity (near 99%) with *Enterobacter* sp. (JF753460.1), *Enterobacter cloacae* (GU459207.1) for *Enterobacter*, and also *Gordonia* sp. (HQ425306.1) and *Gordonia namibiensis* (AF380931.1) for *Gordonia.* So these sequences have been known as *Enterobacter* sp. *and Gordonia* sp. They have been submitted in GenBank with accession numbers JN029956 and JF342357 for

Table 1 Morphological, physiological and biochemical characteristics of strains *Gordonia* sp. and *Enterobacter* sp.

Characters	Results	
	Gordonia sp.	*Enterobacter* sp.
Morphology	Cells are rod shape, Gram positive and orange colored colony	Cells are rod shape, Gram negative and cream colored colony
Motility	Negative	Positive
Catalase	Negative	Negative
Voges-Proskauer	Negative	Positive
Methyl red	Negative	Negative
Nitrate reduction	Positive	Positive
Formation of indole	Negative	Negative
Hydrolysis of starch	Negative	–

Enterobacter sp. MZ-80 and *Gordonia* sp. M01101, respectively.

Electrochemical tests

Figure 1 demonstrates polarization curves for carbon steel in BH medium in the absence and presence of two bacteria after 1 (Fig. 1a) and 2 (Fig. 1b) weeks. Electrochemical corrosion parameters such as corrosion potential (E_{corr}), cathodic (β_c) and anodic (β_a) Tafel slopes, corrosion current density (i_{corr}) and corrosion rate were all derived from polarization curves and are given in Table 2. As can be seen, the presence of bacteria decreased the corrosion potential and increased corrosion current density of carbon steel samples. Also, the cathodic Tafel slope has been increased in the presence of IOB after 2 weeks that shows decrease of oxygen concentration at carbon steel/solution interface. In fact, in the presence of bacteria, alternative processes could occur and affect the cathodic and anodic process activities (Starosvetsky et al. 2001). The activity of strictly IOB, which accumulate on the metal surface with biofilm formation, may lead to significant decrease of oxygen concentration at carbon steel/solution interface. So, it is reasonable to suggest that decrease of oxygen concentration results in a rate reduction of cathodic process and a shift of E_{corr} to negative values during specimen OCP exposure (Starosvetsky et al. 2008).

Figure 2 shows the Nyquist plots for carbon steel specimens in BH medium for an exposure period 1 (Fig. 2a) and 2 (Fig. 2b) weeks without and with two types of the iron bacteria at 30°C. The impedance spectra obtained experimentally were analyzed using Zview2 equivalent circuit software. As seen in Fig. 3 a

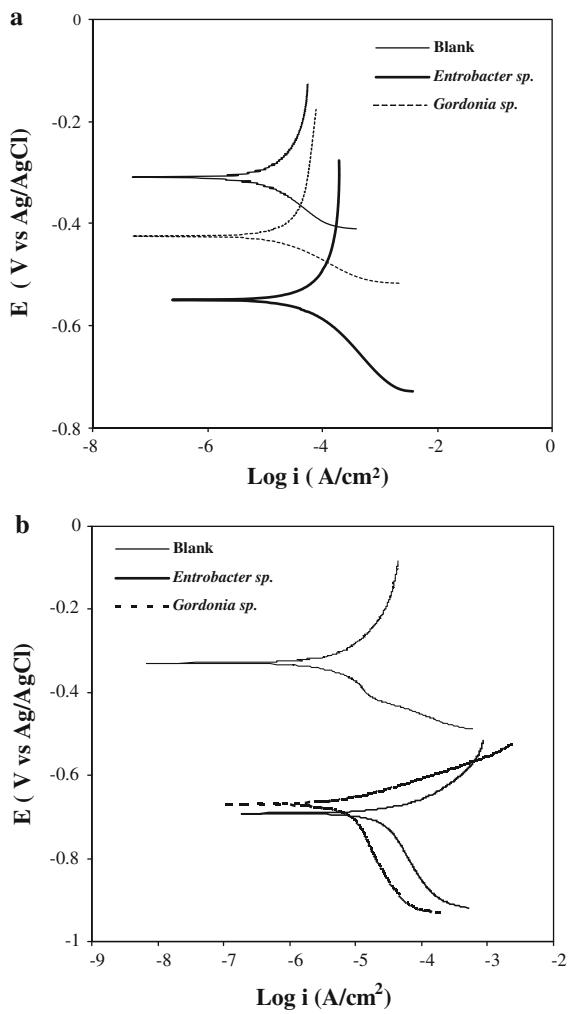

Fig. 1 Potentiodynamic polarization curves for carbon steel in BH medium in the absence and presence of IOB in exposure time: **a** 1 and **b** 2 weeks

Table 2 Polarization parameters for steel in BH medium in the absence and presence of IOB

Sample	E_{corr} (V vs. Ag/AgCl)	$I_{corr} \pm$ SD (μA cm^{-2})	β_c (V/dec)	β_a (V/dec)	Rate (mm/year)
After 1 week					
Blank	−0.305	2.91 ± 0.29	0.075	0.099	0.034
Gordonia sp.	−0.413	5.55 ± 0.18	0.053	0.136	0.065
Enterobacter sp.	−0.531	11.36 ± 0.34	0.077	0.106	0.133
After 2 weeks					
Blank	−0.33	3.23 ± 0.22	0.091	0.094	0.038
Gordonia sp.	−0.67	7.34 ± 0.13	0.285	0.048	0.085
Enterobacter sp.	−0.69	15.14 ± 0.11	0.45	0.046	0.176

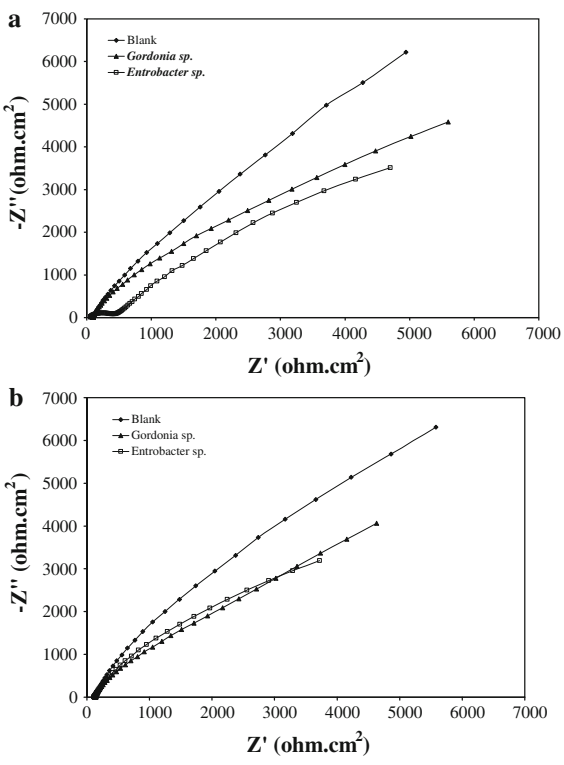

Fig. 2 Nyquist plots for carbon steel in BH medium in the absence and presence IOB in exposure time: **a** 1 and **b** 2 weeks

two-time constant model was used to fit the experimental data that shows the film of corrosion products and the double layer. Data for carbon steel in the sterile medium and presence of two bacteria were analyzed using this equivalent circuit and the quantitative results of impedance measurements are given in Table 3. By comparison of Fig. 2a and b, it is clarified that the impedance magnitudes have been increased in sterile medium due to the formation of a film of

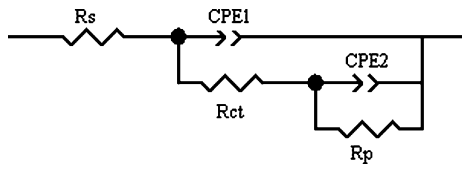

Fig. 3 Equivalent circuit proposed to simulate experimental impedance diagrams in the evaluation of carbon steel in BH medium

corrosion products with a greater thickness than before. But the behavior of IOB indicated a different electrochemical response. The impedance magnitudes have been decreased by exposure time. This modification may indicate that the oxidation mechanisms are different in the absence and presence of bacteria. It is suggested that, the decrease in the R_p can be because of the easy diffusion of electro active species through the biofilm and oxide formed on the surface in the presence of bacteria (Oyasan and Nazir 2010).

Surface analysis

Figure 4 shows the SEM images of the specimens exposed for 1 month in sterile BH medium and BH medium containing IOB. Practically, the whole specimen surface was covered with a dense deposit. But as can be seen, these oxide layers and the pattern of sedimentation on the metal surface are not similar to one another. Deposits covering the corroded sites by *Enterobacter* sp. (Fig. 4c) have a layered structure while the upper layer contains needle-shape aggregate. Deposits that cover the specimens exposed to *Gordonia* sp. are also heterogeneous, plain and form a great fragile oxide layer (Fig. 4b). The cell bodies were not clearly visible on their oxide layer. In sterile

Table 3 Electrical elements obtained from the best fitting of experimental impedance diagrams of the steel/electrolyte interface, using Zview2 program

Sample	R_s (Ω cm^2)	R_{ct} (kΩ cm^2)	$R_p \pm$ SD (kΩ cm^2)	Q_1 (mF cm^{-2})	Q_2 (mF cm^{-2})	$n1$	$n2$
After 1 week							
Blank	85.6	12.83	38.36 ± 0.25	0.76	0.79	0.77	0.71
Gordonia sp.	136.6	2.01	24.57 ± 0.36	0.45	0.66	0.83	0.57
Enterobacter sp.	44.8	0.38	16.65 ± 0.27	0.014	0.77	0.63	0.61
After 2 weeks							
Blank	133.9	4.49	44.34 ± 0.33	0.61	0.39	0.85	0.55
Gordonia sp.	114.4	2.41	12.21 ± 0.15	0.86	0.58	0.81	0.53
Enterobacter sp.	113.2	3.72	8.41 ± 0.16	0.09	0.91	0.79	0.65

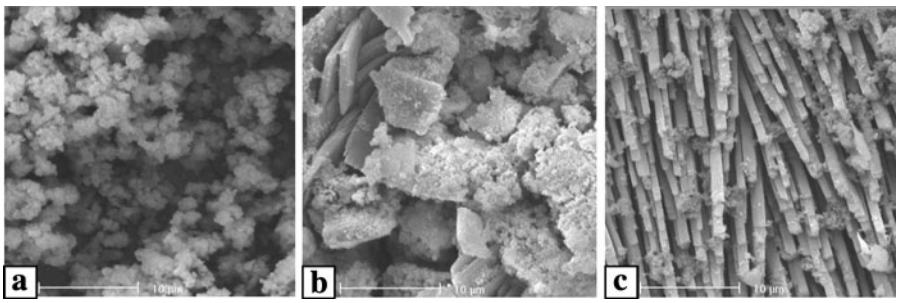

Fig. 4 SEM images of the carbon steel samples exposed for 1 month in BH medium in absence (**a**) and presence of *Gordonia* sp. (**b**) and *Enterobacter* sp. (**c**)

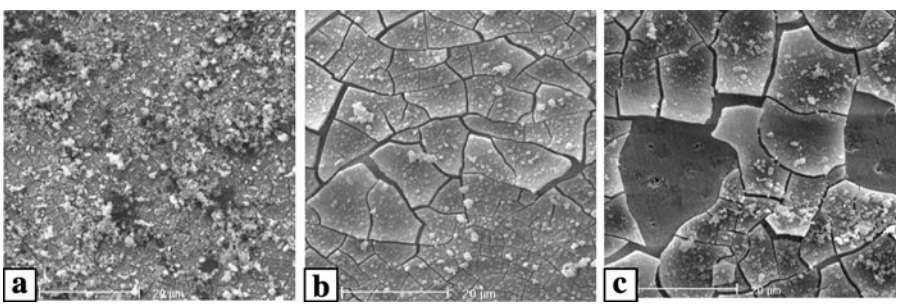

Fig. 5 SEM images of carbon steel samples exposed for 7 days in BH medium in absence (**a**) and presence of *Gordonia* sp. (**b**) and *Enterobacter* sp. (**c**) after polarization

media (Fig. 4a), the carbon steel was covered with a dense, uniform and cauliflower-like layer of deposits. After polarization, the oxide layer still remained on the carbon steel exposed to sterile medium (Fig. 5a). But in the presence of IOB (Fig. 5b, c), this layer was brittle. So cracks and grain boundary attacks have been formed on the carbon steel surfaces especially in the presence of *Entrobacter* sp. (Fig. 5c) that the oxide layer has been removed on the surface and pitting attack happened on the inner layer. In fact, these microorganisms can be detrimental to the integrity of the passive oxide film, and facilitate the local depassivation of the protective layer. Figure 6 shows SEM images of corrosion products on carbon steel that was exposed to BH medium containing the bacteria for 21 days and then the surface was polarized. As is shown, the metal surface was corroded and completely destroyed. The whole surface of the metal was covered with corrosion products.

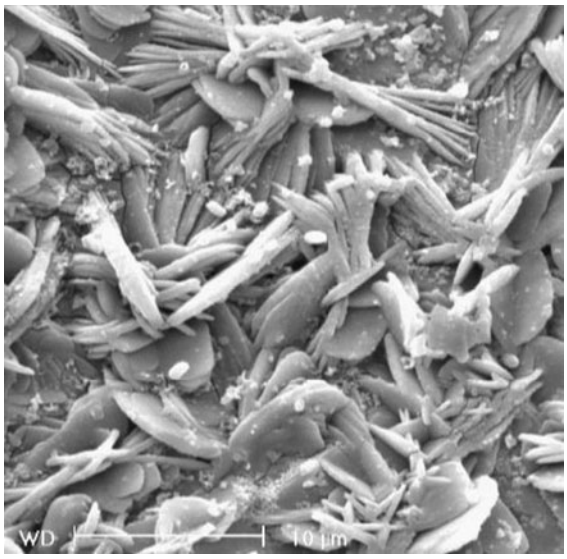

Fig. 6 SEM images of corrosion products on carbon steel that were exposed to BH medium with *Enterobacter* sp. for 21 day and then surface polarized

The X-ray diffraction pattern of the carbon steel corrosion product in sterile media and in the presence of IOB gives some qualitative information about the possible phases that are present. Figure 7 presents the XRD data corresponding to the phase present in the corrosion product samples. The phase identification was carried out by searching and comparing the XRD data with the patterns in the ICDD database. Only one major phase was recognized, namely FeOOH in all of the samples. Table 4 shows the quantitative data of the mentioned compound. It was indicated the peak intensity and width line (d) in the presence of *Enterobacter* sp. and *Gordonia* sp. are different from that in the sterile media. These two parameters are sensitive function of crystal structure. So, it was concluded the different kind of FeOOH structures have been formed on the metal surface in the absence and presence of bacteria. SEM results (Fig. 5) confirmed this difference between structures of oxide layers.

Discussion

In this work, two bacterial strains were isolated from the sewage treatment plants. The main morphological features and the biochemical characteristics of strains were determined. The results indicate that the corrosion process that took place on the metal surface was affected by the presence of these bacteria. These bacteria have the capacity to oxidize ferrous ions to ferric, taking away atomic iron from the structure and turning it into ionic iron, and thus this reduces the mechanical integrity and accelerates the corrosion process. It should be mentioned that bacteria belonging to these genera have been isolated before from wastewater (Pauwels and Verstraete 2006; Yassin et al. 2007; Kim et al. 2003), however, their ability to oxidize iron has not been reported. So, the nobility of these bacteria is because of their ability for oxidation of iron.

By exposure time, E_{corr} and corrosion rate of carbon steel samples has been decreased and increased; respectively, in the presence of IOB. This can be attributed to weakening of steel passivity or surface activation, or decrease of soluble oxygen concentration in solution due to the respiratory activity of iron bacteria during their proliferation (Starosvetsky et al. 2008). In fact, the microenvironment at the metal–liquid interface could be altered by the bacterial films via accumulation of a number of inorganic by-products (Miranda et al. 2006). The change in aggressiveness of the solution at the metal surface was reflected by a reduction in E_{corr}. Some factors such as the cathodic bacterial catalase and the formation of differential aeration cells have been reported for accelerating the anodic metal oxidation reaction or the cathodic oxygen reduction reaction by the bacteria (Yuan and Pehkonen 2007). As a matter of fact, the dense rust deposits formed by IOB may also create oxygen concentration zones and initiate crevice corrosion on carbon steel. Also; visible current increase on anodic curves may result in oxide layer disruption by accumulated corrosion products (Starosvetsky et al. 2001).

There are some possible mechanisms by which microbial metabolic reactions can interfere with the electrochemical reactions at the time that the microorganisms colonize on the metal surfaces (Shi et al. 2003): one of the mechanisms is related to the heterogeneous distribution of the biomasses in biofilms. It is well known that biofilms are heterogeneous and modify the chemical and physical conditions like decreasing the concentration of oxygen near the surface. By consuming or generating substances in biofilms, microorganisms cause concentration gradients of components important for their metabolism which are also important for the

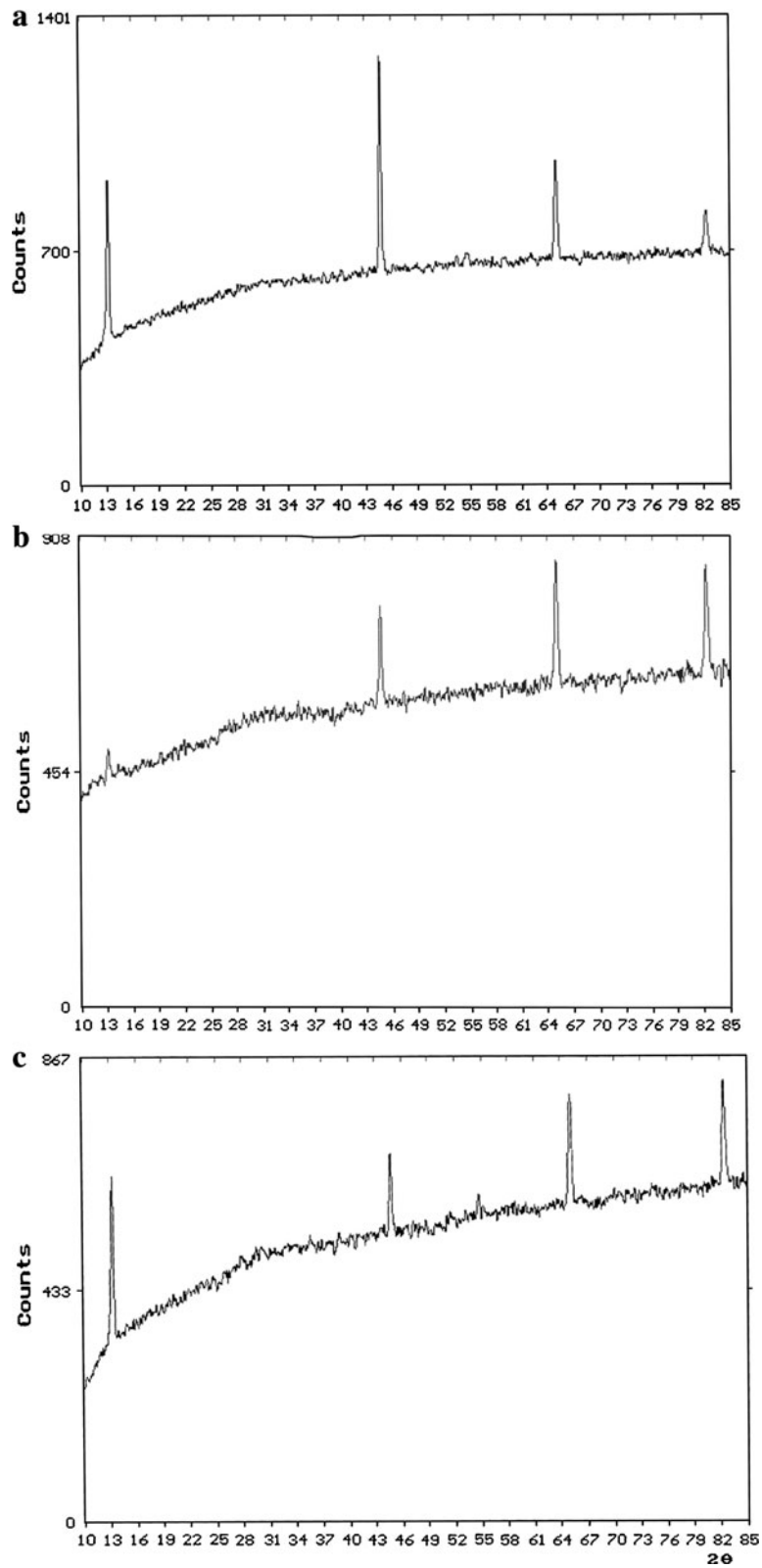

Fig. 7 XRD analysis of carbon steel samples after 1 month in the absence (**a**) and presence of *Gordonia* sp. (**b**) and *Enterobacter* sp. (**c**)

Table 4 The X-ray diffraction intensity of iron oxides compounds in the absence and presence of *Enterobacter* sp. and *Gordonia* sp. after 1 month

Sample	2θ (degree)	*d*-Spacing (nm)	Intensity (a.u.)
Blank	13.23	6.688	482
	44.73	2.024	637
	65.06	1.432	279
	82.37	1.170	130
Gordonia sp.	13.18	6.714	43
	44.69	2.026	191
	65.0	1.434	241
	82.35	1.170	180
	84.59	1.145	31
Enterobacter sp.	13.28	6.662	305
	44.78	2.022	148
	54.72	1.676	46
	65.1	1.432	202
	82.42	1.169	178

electrochemical reactants, e.g., oxygen and protons. Since metabolic activity in biofilms is not uniformly distributed over the colonized surfaces, concentration profiles are formed both vertically and horizontally that may lead to differential concentration cells. In these processes, the microorganisms use the active substrates of electrochemical reaction, as oxygen, and produce other substances that may be used in electrochemical reactions. Such changes in near-surface chemistry have an obvious consequence for electrochemical reactions related to the corrosion.

Under aerobic conditions, corrosion processes are activated by the presence of O_2 (oxidizing agent) in the electrolytic medium. Impedance values were obtained from the formation of a film of corrosion and scale products (Mansfeld and Little 1991). A depressed capacitive loop in the Nyquist plots (Fig. 2b) arises from the time constant of the electrical double layer, called constant phase element (CPE). Use of the CPE, defined by the values of Q and n, is employed in the model to compensate the non-homogeneties in the electrode surface as depicted by depressed nature of the Nyquist semicircle (Nyikos and Pajkossy 1985). The introduction of such CPE is often utilized to interpret data for rough solid electrodes. The impedance, Z, of the CPE is (Kissi et al. 2006):

$$Z_{\mathrm{CPE}} = Q^{-1}(j\omega)^{-n}, \quad (1)$$

where Q stand for the CPE constant, n is depicted as the dispersion parameter and its value being less than the one indicating an imperfect capacitor, $j = (-1)^{1/2}$ is the imaginary unit and ω is the angular frequency in rad s^{-1} ($\omega = 2\pi f$ where f is the frequency in Hz). The values of the double-layer capacitance, C_{dl}, were obtained by using the following equation:

$$f\left(-Z''_{\max}\right) = \frac{1}{2\pi C_{\mathrm{dl}} R_{\mathrm{ct}}}, \quad (2)$$

f is the frequency where the Z'' is the maximum. Aerobic biofilm formation in IOB solution on carbon steel samples at E_{OCP} produces an apparent decrease in R_{P}, in comparison with those obtained in sterile medium, indicating IOB enhanced corrosion. This decline in EIS could be attributed to thinning of the passive layer due to the presence of aerobic IOB. Activity of IOB decreases the concentration of oxygen reaching the electrode surface and restricts access of fresh oxygen to the metal surface. This reduces the formation of fresh oxide by reaction of the metal with dissolved oxygen, and promotes carbon steel corrosion. But in the sterile media, R_{p} increased by exposure time that shows an enhancement in protective characteristics of steel passivity.

The SEM results show the grain boundary and localized attack on the metal surface in the presence of these bacteria. In fact, under aerobic conditions, the microbial colonization usually leads to the formation of differential aeration and concentration cells because of the metabolism of the bacterial colony. The generation of these concentration cells is widely recognized to be detrimental to the integrity of the passive film and to facilitate the initiation of pitting or crevice corrosion, as well. In reality, biofilms tend to create heterogeneous surface conditions that lead to localized corrosion which is usually in the form of pitting (Yuan and Pehkonen 2007). The X-ray diffraction patterns also indicated these bacteria convert ferrous to ferric ions because of gaining energy for growth. But these oxide layers are heterogeneous and affect the corrosion process.

Conclusion

This study investigated the microbiological corrosion of steel by IOB isolated from sewage treatment

plants. It concluded that: (1) Two novel IOB, namely *Gordonia* sp. and *Enterobacter* sp. was isolated and identified by biochemical and molecular methods. The nobility of these bacteria is because of their ability for oxidation of iron. (2) The corrosion potential and corrosion rate was decreased and increased; respectively, in the presence of IOB due to weakening of oxide layer and reduction of oxygen concentration. (3) According to the XRD results and difference of peak intensity and width lines, it was concluded that the different kind of FeOOH was formed on the surface of carbon steel that was also confirmed by SEM results. (4) SEM morphologies have shown that the localized corrosion of samples has been occurred after polarization upon exposure to IOB cultures specially *Enterobacter* sp.

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References

Angell P, White DC (1995) Is metabolic activity by biofilms with sulfate-reducing bacterial consortia essential for long-term propagation of pitting corrosion of stainless steel? J Ind Microbiol 115:329–332. doi:10.1016/S0010-938X(99)00116-X

Atlas RM (1993) Handbook of microbiological media. CRC Press, Boca Raton

Chen YC, Lee CM, Yen SK, Chyou SD (2007) The effect of denitrifying Fe-oxidizing bacteria TPH-7 on corrosion inhibition of sodium molybdate. Corros Sci 49:3917–3925. doi:10.1016/j.corsci.2007.03.042

Dubiel M, Hsu CH, Chien CC, Mansfeld F, Newman DK (2002) Microbial iron respiration can protect steel from corrosion. Appl Environ Microbiol 68:1440–1445. doi:10.1128/AEM.68.3.1440-1445.2002

Fonseca ITE, Feio MJ, Lino AR, Reis MA, Rainha VL (1998) The influence of the media on the corrosion of mild steel by *Desulfovibrio desulfuricans* bacteria: an electrochemical study. Electrochim Acta 43:213–222. doi:10.1016/S0013-4686(97)00227-2

Kim KK, Lee CS, Kroppenstedt RM, Stackebrandt E, Lee ST (2003) *Gordonia sihwensis* sp. nov., a novel nitrate-reducing bacterium isolated from a wastewater-treatment bioreactor. Int J Syst Evol Microbiol 53:1427–1433. doi:10.1099/ijs.0.02224-0

Kissi M, Bouklah M, Benkaddour M (2006) Establishment of equivalent circuits from electrochemical impedance spectroscopy study of corrosion inhibition of steel by pyrazine in sulphuric acidic solution. Appl Surf Sci 252:4190–4197. doi:10.1016/j.apsusc.2005.06.035

Lee AK, Newman DK (2003) Microbial iron respiration: impacts on corrosion processes. Appl Microbiol Biotechnol 62:134–139

Mansfeld F, Little B (1991) A technical review of electrochemical techniques applied to microbiologically influenced corrosion. Corros Sci 32:247–272. doi:10.1016/0010-938X(91)90072-W

Miranda E, Bethencourt M, Botana FJ, Cano MJ, Sanchez-Amaya JM, Corzo A, de Lomas, JG, Fardeau ML, Ollivier B (2006) Biocorrosion of carbon steel alloys by an hydrogenotrophic sulfate-reducing bacterium *Desulfovibrio capillatus* isolated from a Mexican oil field separator. Corros Sci 48:2417–2431. doi:10.1016/j.corsci.2005.09.005

Morgan E (1993) Chemometrics: experimental design. Analytical chemistry by open learning. Wiley, New York

Nyikos L, Pajkossy T (1985) Electrochemical and in situ Mössbauer studies of tin passivation. Electrochim Acta 30:529–533. doi:10.1016/0013-4686(85)80043-8

Olesen BH, Avci R, Lewandowski Z (2000) Manganese dioxide as a potential cathodic reactant in corrosion of stainless steels. Corros Sci 42:211–227. doi:10.1016/S0010-938X(99)00071-2

Oyasan N, Nazir H (2010) The influence of *Pseudomonas aeruginosa* on corrosion behavior of nickel-cobalt alloy. Commun Fac Sci Univ Ankara Ser B 56:1–9

Pauwels B, Verstraete W (2006) The treatment of hospital wastewater: an appraisal. J Water Health 04:405–416. doi:10.2166/wh.2006.025

Rajasekar A, Babu TG, Pandian SK, Maruthamuthu S, Palaniswamy N, Rajendran A (2007) Biodegradation and corrosion behavior of manganese oxidizer *Bacillus cereus* ACE4 in diesel transporting pipeline. Corros Sci 49:2694–2710. doi:10.1016/j.corsci.2006.12.004

Rao TS, Kora AJ, Anupkumar B, Narasimhan SV, Feser R (2005) Pitting corrosion of titanium by a freshwater strain of sulphate reducing bacteria (*Desulfovibrio vulgaris*). Corros Sci 47:1071–1084. doi:10.1016/j.corsci.2004.07.025

Seneath PHA, Mair NS, Sharpe ME, Holt JG (1986) Bergey's manual of systematic bacteriology, 9th edn. Williams and Wilkins, Baltimore

Shi X, Avci R, Geiser M, Lewandowski Z (2003) Comparative study in chemistry of microbially and electrochemically induced pitting of 316L stainless steel. Corros Sci 45:2577–2595. doi:10.1016/S0010-938X(03)00079-9

Starosvetsky D, Armon R, Yahalom J, Starosvetsky J (2001) Pitting corrosion of carbon steel caused by iron bacteria. Int Biodeter Biodegrad 47:79–87. doi:10.1016/S0964-8305(99)00081-5

Starosvetsky J, Starosvetsky D, Pokroy B, Hilel T, Armon R (2008) Electrochemical behaviour of stainless steels in media containing iron-oxidizing bacteria (IOB) by corrosion process modeling. Corros Sci 50:540–547. doi:10.1016/j.corsci.2007.07.008

Teng F, Guan YT, Zhu WP (2008) Effect of biofilm on cast iron pipe corrosion in drinking water distribution system: corrosion scales characterization and microbial community structure investigation. Corros Sci 50:2816–2823. doi:10.1016/j.corsci.2008.07.008

Unas EJ, Ramanauskas R, Lugauskas A, Samulevicien M, Leinartas K (2005) Microbially influenced corrosion

acceleration and inhibition. EIS study of Zn and Al subjected for two years to influence of *Penicillium frequentans*, *Aspergillus niger* and *Bacillus mycoides*. Electrochem Commun 7:305–311. doi:10.1016/j.elecom.2005.01.012

Valcarce MB, de Sanchez SR, Vazquez M (2006) Brass dezincification in a tap water bacterial suspension. Electrochim Acta 51:3736–3742. doi:10.1016/j.electacta.2005.10.034

Xu C, Zhang Y, Cheng G, Zhu W (2007) Localized corrosion behavior of 316L stainless steel in the presence of sulfate-reducing and iron-oxidizing bacteria. Mater Sci Eng A 443:235–241. doi:10.1016/j.msea.2006.08.110

Yassin AF, Shen FT, Hupfer H, Arun AB, Lai WA, Rekha PD, Young CC (2007) *Gordonia malaquae* sp. nov., isolated from sludge of a wastewater treatment plant. Int J Syst Evol Microbiol 57:1065–1068. doi:10.1099/ijs.0.64893-0

Yuan SJ, Pehkonen SO (2007) Microbiologically influenced corrosion of 304 stainless steel by aerobic *Pseudomonas* NCIMB 2021 bacteria: AFM and XPS study. Colloid Surf B Biointerfaces 59:87–99. doi:10.1016/j.colsurfb.2007.04.020

Yuan SJ, Choong AMF, Pehkonen SO (2009) AFM study of microbial colonization and its deleterious effect on 304 stainless steel by *Pseudomonas* NCIMB 2021 and *Desulfovibrio desulfuricans* in simulated seawater. Corros Sci 51:1372–1385. doi:10.1016/j.corsci.2009.03.037