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Corrosion behavior of low-alloy steel in the presence of *Desulfotomaculum* sp.

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ABSTRACT

The objective of this study was to determine the effect of sulfate-reducing *Desulfotomaculum* sp. bacteria isolated from a crude oil field on the corrosion of low-alloy steel. The corrosion rate and mechanism were determined with the use of Tafel slopes, mass loss method and electrochemical impedance spectroscopy (EIS). The formation of the biofilm and the corrosion products on the steel surface was determined with scanning electron microscopy (SEM) micrographs and energy dispersive X-ray spectra (EDS) analysis. It was observed from the Tafel plots that the corrosion potential exhibited a cathodic shift that verifies an increase in the corrosion rates. The semicircles tended to open at lower frequencies in the Nyquist plots which indicates the rupture of the protective film. The corrosion current density reached its maximum value at the 14th hour after the inoculation and decreased afterwards. This was attributed to the accumulation of corrosion products on the surface.

1. Introduction

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms, which carry out dissimilatory reduction of sulfate to H_2S. SRB grow well in anaerobic niches, such as soil sediments, oil fields and wastewater treatment plants. They induce biocorrosion on the industrial equipment, such as pipes, pumps and tanks located in these environments [1–4]. Production of the extracellular polymeric substances (EPS) facilitates colonization of metal surfaces by SRB and formation of biofilms [5–7]. Suggested models of SRB-induced corrosion mechanisms include the formation of the biofilms, which affect the electrochemical characteristics of the metal surfaces, bound metal ions and the corrosion products, such as iron sulfides [6,8–10]. Other mechanisms, which are used to explain SRB contribution to the corrosion process, are cathodic depolarization via hydrogenases; release of the corrosive bacterial metabolites, such as hydrogen sulfide; production of the volatile phosphorus compounds and degradation of the protective films [11–15].

In the oil, gas and shipping industries, the activities of SRB are particularly serious, which result in high corrosion costs [16–18]. The activity of SRB generally causes pitting corrosion of metal equipment. However, stress corrosion cracking and crevice corrosion are also reported for different metals [19–21].

The present study was carried out with *Desulfotomaculum* sp. isolated from a crude oil production field having serious corrosion problems. The corrosive activity of an anaerobic SRB strain on low-alloy steel, widely used in the petroleum industry, was investigated with electrochemical impedance spectroscopy (EIS) complemented by scanning electron microscopic images.

2. Materials and methods

The SRB strain used for the study was isolated from an oil–water mixture taken from an oil production well in Batman, Turkey [22]. The composition of the Medium 63a used for enrichment and cultivation of the SRB was: Solution A: 5.0 g sodium pyruvate ($CH_3COCONa$) + 2.0 g sodium acetate (CH_3COONa) + 2.0 g $MgSO_4{\cdot}7H_2O$ + 1.0 g NH_4Cl + 1.0 g Na_2SO_4 + 1.0 g yeast extract + 0.5 g K_2HPO_4 + 0.1 g $CaCl_2{\cdot}2H_2O$ + 1.0 mg resazurin ($C_{12}H_6NNaO_4$) + 980 mL distilled water; Solution B: 0.5 g $FeSO_4{\cdot}7H_2O$ + 10 mL distilled water; Solution C: 0.1 g ascorbic acid ($C_6H_8O_6$) + 0.1 g sodium thioglycolate ($HSCH_2COONa$) + 10 mL distilled water. Solution A was boiled for a few minutes and cooled under N_2 atmosphere. Solutions B and C were added and the pH was adjusted to 7.0 with 0.1 M NaOH [23]. Solution B was prepared with 0.004 g $FeSO_4{\cdot}7H_2O$ for electrochemical measurements. Cell growth was monitored from microscopic counts in the Petroff–Housser chamber [24]. The stock culture was maintained in a medium under nitrogen atmosphere at 4 °C. The isolate named 1A1-2 uses the following compounds as carbon sources in the presence of sulfate: butyrate, glucose, lactate, propionate and pyruvate. The isolate is spore-forming (spherical, 0.7–1.6 μm in diameter), motile and gram negative. It grows optimally at 30–32 °C and pH 6.8–7.2. The cells are curved rods shaped with an average size of 0.47 ± 0.06 × 5.05 ± 1.48 μm. The isolate resembles the *Desulfotomaculum* sp. physiologically.

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The composition of the low-alloy steel obtained from the oil production field is as follows: 99.105% Fe, 0.113% C, 0.332% Mn, 0.106% Ni, 0.01% P, 0.024% S, 0.21% Si, 0.05% Cr, and 0.05% Cu. There were steel coupons, which were cut from raw material for the mass loss measurements. The coupons were polished with 1200-grit emery paper, rinsed with double-distilled water, degreased with acetone and then air-dried. Their total areas and initial masses were determined before the corrosion tests. The test coupons were placed in N_2 flushed Hungate tubes (Bellco Glass Inc.) and sterilized by autoclaving. After that, 10 mL of sterile medium was added and the medium was inoculated with a 24 h grown culture with an initial concentration of 10^7 cell/mL. The Petroff–Housser counting chamber was used to count bacteria. Before the experiments, a bacteria sample was taken from the culture bottle and the number of bacteria was determined with the counting chamber. Based on these results, the necessary calculations were made to determine how many ml should be used to achieve this concentration in the cell for the electrochemical experiments.

The tubes were opened at the end of a 1-month incubation period at 30 °C. The coupons were taken out, cleaned and weighed. Each experiment was carried out in triplicate and the average of three values was taken. The value of the corrosion rate based on mass loss was calculated from the following equation [25]

$$v = (m_1 - m_2)/S \times t \quad (1)$$

where m_1 is the mass of the specimen before corrosion (mg), m_2 is the mass of the specimen after corrosion (mg), S is the total area of the specimen (cm^2), t is the exposure time (h) and v is the corrosion rate ($mg\,cm^{-2}\,h^{-1}$). The total area of each coupon was determined for every experiment, because two coupons may be identical in mass, but different in shape and surface area. The increase in the surface area means more area on which the bacteria can bind. Therefore, the surface area has a significant effect upon the corrosion rate.

The surface analysis of the coupons was performed with a scanning electron microscope. The coupons were taken out and cleaned after a 1 month exposure to the medium with and without SRB. The cleaned coupons were coated with gold (Polaron SC 502 sputter coater) for the SEM examination. Another coupon with SRB biofilm was immersed overnight in a 2.5% glutaraldehyde solution to fix the SRB biofilm to the surface [16]. The coupon was then washed two times with distilled water. This was followed by dehydration with the use of a graded series of ethanol (70%, 80%, 90% and 100%). The coupon was then immersed in amyl acetate, subjected to critical point drying with CO_2 (Polaron, CPD 7501) and coated with gold for the SEM examination. The scanning electron micrographs were taken at 15 kV with the Jeol JSM 6060. The energy dispersive X-ray spectra analyses (EDS) were obtained with the IXRF-EDS 2000 Microanalysis system of the Jeol JSM 5600 SEM.

Table 1
The corrosion rates of the steel coupons in the sterile culture medium and *Desulfotomaculum* sp. inoculated culture medium after 1 month [22].

Culture medium	Corrosion rate ($\mu g\,cm^{-2}\,h^{-1}$)
Sterile (without bacteria)	4.3 ± 0.9
Inoculated with *Desulfotomaculum* sp.	18.1 ± 2.8

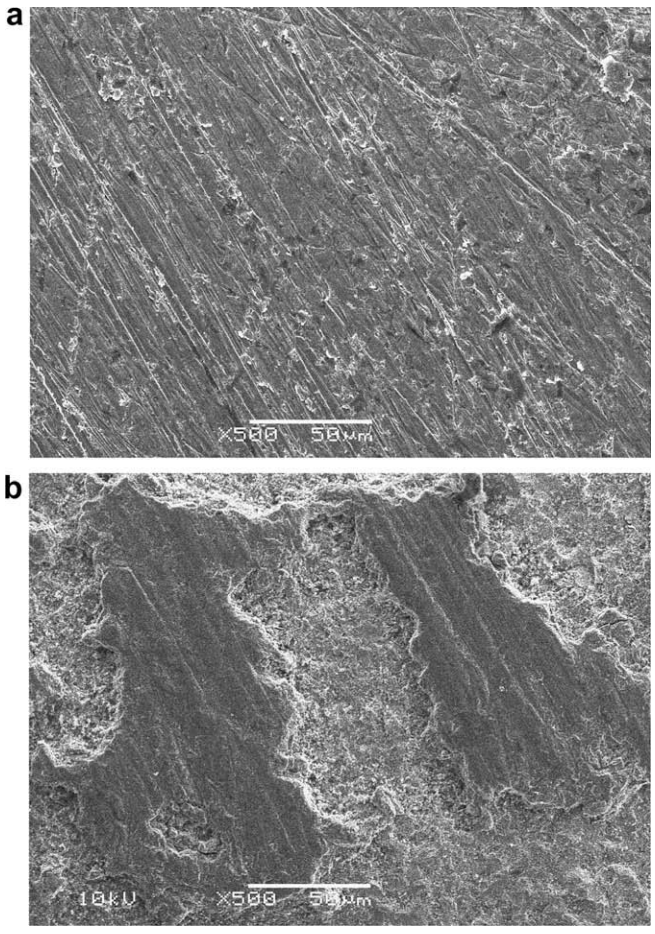

Fig. 1. The SEM micrograph of the surface of the steel coupon exposed to (a) sterile culture medium and (b) culture medium with *Desulfotomaculum* sp. (after the removal of corrosion products).

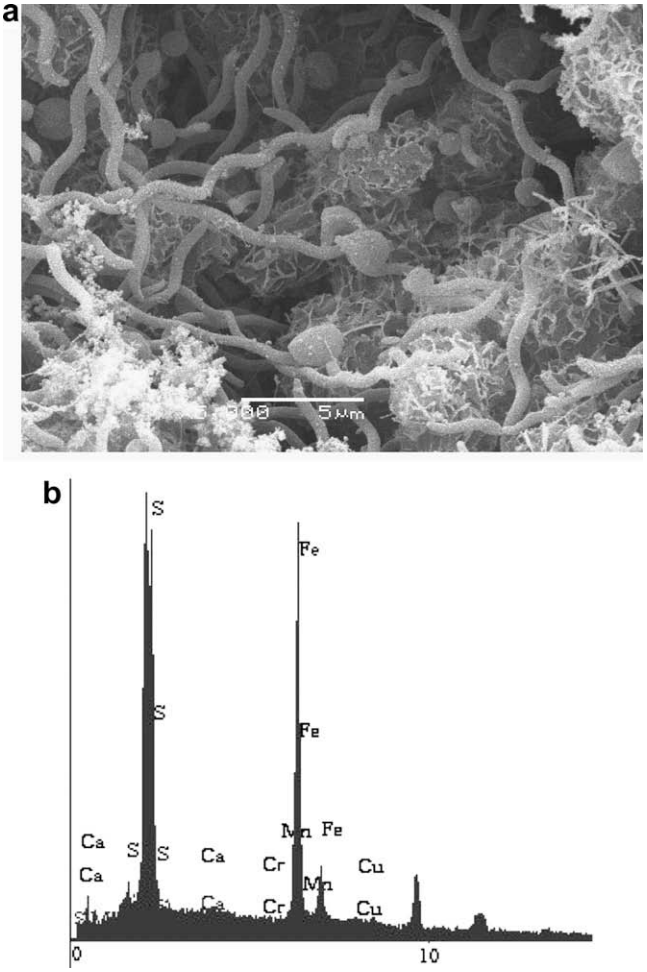

Fig. 2. (a) The SEM micrograph and (b) The EDS spectrum of the steel coupon after immersion for 1 month in the culture medium with *Desulfotomaculum* sp.

A steel electrode with a 4 mm diameter embedded in methyl methacrylate resin was used as the working electrode for the electrochemical measurements. Pretreatment of the steel surface was carried out by grinding with a 1200-grit emery paper and rinsing with double-distilled water. This grit size was used by keeping in mind that it was impossible to find smooth-surfaced pipes at the crude oil production site. It is also a known fact that if the surface becomes too smooth, then bacteria cannot attach to it.

The electrochemical studies were carried out with a computerized CHI Instrument 660 B system in a conventional three-electrode cell. A platinum wire was used as the auxiliary electrode.

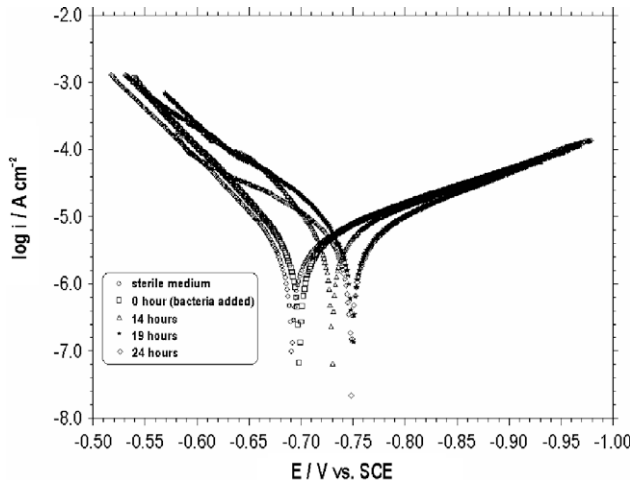

Fig. 3. The polarization curves of low-alloy steel in sterile and *Desulfotomaculum* sp. inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$.

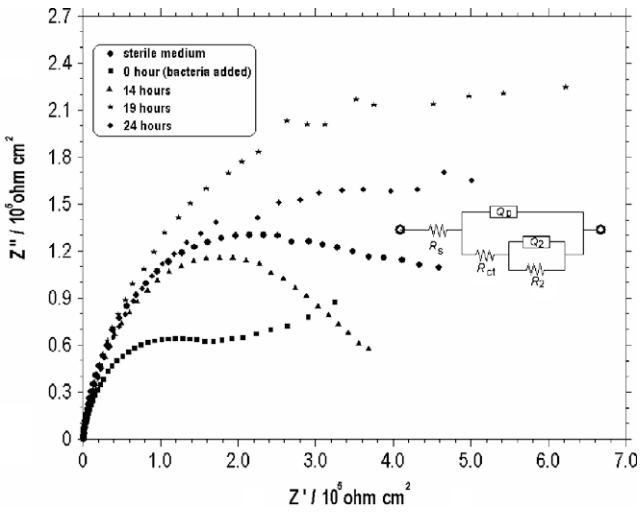

Fig. 4. The EIS and equivalent circuit of low-alloy steel in the sterile and *Desulfotomaculum* sp. inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$.

The reference electrode was a saturated calomel electrode (SCE). All solutions were deaerated for 30 min. with pure argon and mixed with a magnetic stirrer. All of the measurements were made at 25 °C (room temperature). The polarization tests were conducted in Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$. The electrochemical measurements were carried out in media with a low Fe content, because the preliminary experiments showed that there was an extensive FeS production in the Fe rich media, which deposit on the surface and inhibit the current. The cell components were washed with 70% ethanol several times and kept in alcohol to ensure sterility. The cell was then rinsed with sterile double-distilled water, filled with sterile medium and sealed to avoid contamination. Since the medium was anaerobic and the duration of the experiment was kept short (limited to 24 h) there was a minimal risk of contamination by other bacteria.

The corrosion behavior of the steel sample was determined in a sterile medium and after the inoculation of the microbial culture. The isolate was repeatedly transferred into fresh Medium 63a prepared with 0.004 g/L of $FeSO_4{\cdot}7H_2O$ to acclimatize the isolate to low ferrous concentrations. The electrochemical cell was inoculated with these acclimatized cultures. The measurements for the sterile medium were made at 0, 14, 19 and 24 h after the inoculation of the SRB. The electrochemical polarization or Tafel curves were recorded by scanning the potential −200 and +200 from the open circuit potential (OCP) at a sweep rate of 5 mV s^{-1}. The EIS measurements were made at open circuit potential using a 5 mV amplitude sinusoidal signal over frequencies ranging from 100,000 to 0.02 Hz.

3. Results and discussion

The corrosion rates of the steel coupons without the bacteria and with *Desulfotomaculum* sp. calculated from the Eq. (1) are shown in Table 1. The corrosion rates at the end of an incubation period of thirty days are approximately four times higher for *Desulfotomaculum* sp. inoculated medium compared to the sterile medium.

Fig. 1a and b show the SEM micrographs of the steel coupons exposed for one month to the sterile culture medium without *Desulfotomaculum* sp. and with *Desulfotomaculum* sp., respectively. There was no sign of corrosion on the metal surface after 1 month when the steel coupon was in the sterile medium (Fig. 1a). There were scratches on the surface of the sterile coupon formed due to the grinding of the coupon with emery paper. However, there were large craters and pits on the inoculated coupon after the removal of the corrosion product. It was apparent that *Desulfotomaculum* sp. in culture medium induces serious corrosion on the steel coupons.

Fig. 2a illustrates the biofilm formation on the steel coupon surface immersed in the culture medium with *Desulfotomaculum* sp. for 1 month. The SEM micrograph revealed the presence of the corrosion products, cells, spores and EPS fibers distributed over the coupon. The EDS analyses of the corrosion products on coupons were illustrated in Fig. 2b. Analysis of the corrosion products on the coupon surface revealed extensively large S peaks, besides the Fe peak.

Table 2
The corrosion parameters determined from the Tafel plots for low-alloy steel in the sterile and *Desulfotomaculum* sp. inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$.

	Sterile medium	Just after the inoculation	14 h after the inoculation	19 h after the inoculation	24 h after the inoculation
E_{cor},V vs SCE	−0.691	−0.698	−0.730	−0.750	−0.750
Cor. current (μA)	0.483	0.483	1.037	0.891	0.741
I_{cor} (μA cm^{-2})	3.85	3.85	8.20	7.10	5.90
Cathodic tafel slope β_c (V dec^{-1})	0.194	0.185	0.19	0.168	0.158
Anodic tafel slope β_a (V dec^{-1})	0.067	0.062	0.103	0.096	0.13

Table 3
The fitting parameters of the EIS for low-alloy steel in the sterile and *Desulfotomaculum* sp. inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$.

	Sterile medium	Just after the inoculation	14 h after the inoculation	19 h after the inoculation	24 h after the inoculation
R_s ($\Omega\,cm^2$)	817.9	850.9	901	817.9	817.9
$R_{ct} \times 10^{-5}$ ($\Omega\,cm^2$)	3.4	1.57	2.9	4.8	3.6
n_p	0.8	0.78	0.8	0.78	0.79
$Y_p^o \times 10^6$ ($\Omega^{-1}\,cm^{-2}$)	1.8	0.48	1	0.91	1.2
$R_2 \times 10^{-5}$ ($\Omega\,cm^2$)	2.1	3.5	2	4.6	2.35
n_2	0.75	0.49	0.44	0.73	0.89
$Y_2^o \times 10^5$ ($\Omega^{-1}\,cm^{-2}$)	2.65	0.9	1.6	0.73	1.1

The Tafel plots of the polarization curves from the steel electrodes immersed in the sterile and inoculated medium after incubation periods of 14, 19 and 24 h are shown in Fig. 3. Values of the electrochemical corrosion parameters (corrosion potential (E_{cor}), corrosion current density (I_{cor}), cathodic and anodic Tafel slopes) are shown in Table 2. Inoculation of the *Desulfotomaculum* sp. to the medium caused initially a small shift in corrosion potential to more cathodic values compared to the sterile medium. The E_{cor} value shifted considerably towards more cathodic values with increased incubation time, which means the increased rate of corrosion. The decrease in the anodic and cathodic Tafel slopes was attributed to the ease of the respective processes [26]. However, the values listed in Table 2 show that the anodic Tafel slope values increased and the cathodic Tafel slope values decreased with the inoculation time. This shows the presence of a mixed control process.

Fig. 4 shows the EIS and equivalent circuit used in the analysis of low-alloy steel in the sterile and inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$.

The fitting parameters of the EIS for low-alloy steel in the sterile and *Desulfotomaculum* sp. inoculated Medium 63a containing 0.004 g/L of $FeSO_4{\cdot}7H_2O$ are given in Table 3. Different models for the equivalent circuit used in the related articles in the literature were examined. The best fitting circuit is presented. R_s denotes the resistance of solution, which is similar for the sterile or inoculated medium. R_{ct} is the resistance of film formed on the surface of steel and R_2 is the charge transfer resistance. Q_2 is the constant phase element (CPE) of the electric double layer and Q_p is the CPE of the surface film, n is depicted as the dispersion parameter and its value being less than the one indicating an imperfect capacitor. Increase of the roughness or the lateral and vertical heterogeneity of the surface film means the decrease of the conductivity and n value [26–28].

The inspection of R_2 related to the resistance of the biofilm formed upon the surface shows that it increases from $2.1 \times 10^5\ \Omega\,cm^2$ in the sterile medium to $3.5 \times 10^5\ \Omega\,cm^2$ after the inoculation of the bacteria. However, this value shows a marked decrease at the 14th hour after the inoculation and increases afterwards. This may be explained by the increased accumulation of the metabolic by-products onto the surface. The increase in the R_2 values after a certain time may be attributed to the increased porosity of the biofilm.

The increase in charge transfer resistance (R_2) causes the increase in the potential of the capacitive component parallel to it. This eventually leads to an increase in the corrosion rate. Similarly, the decrease in the R_{ct} has the same effect that enables the easy diffusion of electroactive species through the biofilm formed on the surface of the steel electrode at the 14th hour, which seems to control the corrosion of low-alloy steel [29]. A similar equivalent circuit model (with capacitance instead of CPE) was also used by Galvan-Martinez et al. for the analysis of the EIS data of X52 steel immersed in Postgate C media inoculated with SRB at day 1 and 2 [29]. They observed an increase in the charge transfer resistance value for the first two days, and then the value decreased at the 4th day. In our study, this value decreased in an earlier incubation period compared to their bacteria. The SRB species used in this study may be physiologically more active than their SRB strain. Since depressed semicircles are frequently observed, as in the case of Hernández-Gayosso et al., in the present study, a constant phase element (CPE) was preferred as a substitute for a capacitor to compensate for the deviations observed on actual systems [30].

The increase in the dimensions of the capacitive loops indicates the formation of an FeS layer on the surface. However, the nonclosing behavior of the loops shows that the layer has a porous structure [31]. This was also verified by the fact that the potential values remained almost constant at prolonged incubation periods.

According to Liu et al. [10] the ferrous ions (Fe^{2+}) produced by the electrode process react with sulfide metabolized by the bacteria-forming iron sulfides (FeS_x). Therefore, the structure of the biofilm changes according to the different growth phases of the SRB. Our findings also support this fact.

4. Conclusions

Desulfotomaculum sp. isolated from an oil field caused corrosion in low-alloy steel under laboratory conditions. The surface analysis revealed the role of iron sulfides in the *Desulfotomaculum* sp. induced corrosion process. The bacteria were observed to affect the corrosion potential and corrosion current density, which enhanced the corrosion rate within the first 24-hour period following incubation. The incubation of the SRB in culture medium accelerates the cathodic depolarization process of low-alloy steel, but slows down the anodic process. The analysis of the Nyquist diagrams with the use of equivalent circuits showed the initiation of biofilm formation after a certain incubation period, which affected the resistance values of the steel surface. The corrosion products (most probably iron sulfides) started to affect the biofilm after a certain incubation period, caused by the enlargement of the capacitive loops displaying nonclosing behavior.

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