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Investigation Of Active Corrosion Sites Of Biofilm Formed At Mild Steel Electrolyte Interface Induced By Mixed Bacterial Culture

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Abstract: Biocorrosion is a complicated process that can result in unexpected corrosion disaster. Real time detection of Microbial Induced or (Enhanced) Corrosion (MIC) effects became an urgent request. Therefore, any information on the chemical structure of corrosion products and microbiological deposits, as well as electrochemical polarization can be extremely useful in applications. In this study, SEM, FTIR and potentiodynamic technique have been used to investigate biocorrosion caused by *Pseudomonas marginalis* and *Desulfomonas pigra* in single and mixed cultures. The results showed that the mild steel suffered from general corrosion in single rather than that in mixed cultures.
Keywords: *P. marginalis, D. pigra*, MIC, biocorrosion SEM, electrochemical.

INTRODUCTION:

Corrosion is an electrochemical process involving an anodic reaction involving the oxidation of the metal, and a cathodic reaction based on the reduction of a chemical species. These reactions can be enhanced by microbial activities that accelerate the rate of anodic and/or cathodic reactions[1], especially when the organisms are in close contact with the metal surface forming a biofilm. The resulting metal deterioration is known as biocorrosion, or microbially-influenced corrosion (MIC) [2]. The MIC is the damage caused or accelerated by the presence of microorganisms and their metabolic activities[3]. If the microorganisms are in the aqueous solution, they first attach to the surface and then grow, replicate and produce exopolymers (EPS), forming a cohesive structure known as a biofilm[4]. This process depends on the surface characteristics of substrates, including metal surface free energy, roughness, hydrophobicity, and metallurgical features[5]. In other words, the biocorrosion is the result of the synergetic interactions of the metal surface, abiotic corrosion products, bacterial cells and cells metabolites[2]. MIC does not produce a defined type of damage; however, it mostly results in a localized type of corrosion that manifests in pitting, crevice corrosion, under-deposit corrosion, cracking, enhanced erosion corrosion, and dealloying [(6, 7, 8)]. Practically, MIC is really the result of synergistic interactions of different microbes, consortia that coexist in the environment and are able to affect the electrochemical processes through co-operative metabolisms [(9, 10)]. Various mechanisms of biocorrosion, which reflect the variety of physiological activities carried out by different types

of microorganisms, have been identified; however, it must be remembered that, in nature, these microbial processes do not act in isolation, but in concert with the chemical and electrochemical forces in the particular environment[9].

There are no official figures for the cost of MIC, but some indication of its importance can be gained from individual companies or sectors of industry. It has been estimated that 20-50% of all corrosion damage to metallic materials is microbially influenced [11, 12]. The study in the United States estimated the direct costs of corrosion to be approximately 4.9% of the gross national product (GNP) for an industrialized nation[13]. In Egypt, the petroleum industries suffer from corrosion induced by microorganisms. It was estimated that one Company (Gulf of Suez Petroleum Co., GUPCO) spends more than $ 1 million per year to combat MIC[14].

The electrochemical reactions involved in the corrosive processes caused by microorganisms can be clarified by obtaining information on the chemical structure of corrosive products and microbiological layers, as well as by quantifying the corrosion rate[15] through electrochemical investigations[6].

In this work, we investigated the simultaneous and mutual effect of the bacterial isolates *P. marginalis* and *D. pigra* in single and mixed cultures on the corrosion behavior of mild steel as well as, the formation of corrosion products/biofilm on the mild steel surface examined using scanning electron microscopy (SEM), FT-IR spectroscopy and Tafel polarization curves (an electrochemical technique).

MATERIALS AND METHODS:

Cultures, growth conditions and characterization:

Pseudomonas marginalis and *Desulfomonas pigra* were isolated from formation water that has the following analysis (TDS, 45100 ppm; Na^+, 13640 ppm; K^+, 500 ppm; Ca^{2+}, 240 ppm; Mg^{2+}, 4656 ppm; Fe^{2+}, 0.11 ppm; Sr^{2+}, 8 ppm; Cl^-, 28400 ppm; SO_4^{2-}, 4100 ppm; HCO_3^-, 135 ppm and CO_3^{2-}, 54 ppm), supplied by Al-Amal Petroleum Company (Amapetco), Egypt and municipal sewage water, respectively.

P. marginalis was isolated and purified on a basal medium[16] which was prepared as follows (g/l): KH_2PO_4, 3; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.2; $(NH4)_2SO_4$, 0.5; $FeSO_4.7H_2O$, trace; Na_2S, 10; Glucose, 5.0; Bromocresol purple, trace and Agar, 20 and the pH was adjusted at 7.0 ± 0.2.

D. pigra was isolated and purified on Postgate medium B[17] which was prepared as follows (g/l): KH_2PO_4, 0.5; NH_4Cl, 1; $CaSO_4$, 1; $MgSO_4.7H_2O$, 2; Sodium lactate, 3.5; Yeast extract, 1; Ascorbic acid, 0.1; Thioglycollic acid, 0.1; $FeSO_4.7H_2O$, 0.5 and Agar, 20 and the pH was adjusted at 7.0 ± 0.2. Both bacterial isolates were purified using streaking method[18]. *D. pigra* was cultured under anaerobic conditions using alkaline pyrogallol (0.5% in 50% potassium hydroxide solution) in Conway plate[19] as shown in Figure (1) where black colonies indicate sulfate reducing bacteria.

Both isolates were characterized and identified based on Bergey's manual of determinative bacteriology, 9th edition[20]. *P. marginalis* identification was confirmed using Biolog identification GN2 MicroPlateTM System (Biolog, Inc., Hayward,USA) according to standard protocols[21].

Test medium and culture conditions:

The bacterial isolates, individually and in mixture, were transferred into 200 ml glass jars, containing 50 ml of sterile GYM medium (recommended for growth of both isolates) which was prepared as follows (g/l): K_2HPO_4, 0.1; $(NH_4)_2SO_4$, 0.5; $FeSO_4.7H_2O$, 0.5; Glucose, 1; Yeast extract, 25 and Agar, 20 and the pH was adjusted at 7.0 ± 0.2. A set of three coupons of mild steel under investigation, chemically analyzed by the Central Metallurgical R & D Institute (CMRDI), Egypt, and had the following composition (%): C, 0.0165; Si, 0.0009; Mn, 0.228; P, 0.0114; S, 0.00668; Al, 0.0226; Co, 0.00431; V, 0.0005; Pb, 0.001; Cu, 0.023; Cr, 0.00993; Mo, 0.0008; Ni, 0.019; Nb, 0.0005; Ti, 0.00032; W, 0.0204; As, 0.00136; B, 0.00017 and Fe, 99.64 were immersed in each jar after grinding with P320 and P1000 emery papers, degreasing with acetone and ethanol[22, 23] and drying in air. The coupons had different sizes; 1 x1 x 0.2, 2 x 2 x 0.2 and flag-shaped 1 x 1 x 0.2 cm for scanning electron microscopic (SEM), Fourier-transform infrared spectroscopy (FTIR) and electrochemical study, respectively. Jars containing bacteria and coupons were incubated at 30°C for 21 days. A control test with coupons in sterile medium was run at the same conditions.

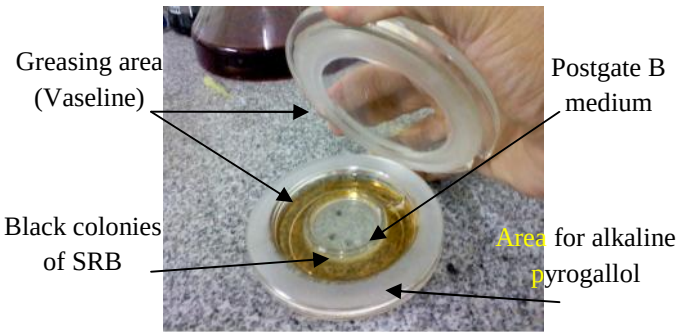


Figure (1): Conway plate used for SRB purification. The plate contained the inner area used for SRB growth at which Postgate B was poured in, the outer for alkaline pyrogallol (reducing agent) and the plate lips for greasing.

Scanning electron microscope (SEM) and FT-IR spectroscopic analysis of the coupons surface:

After the incubation period, the biofilm on the surface of the coupons (1 x 1 x 0.2 cm) were fixed by immersing for 15 min in a 4% glutaraldehyde solution and then dehydrated using four ethanol solutions (15 min each): 25, 50, 75 and 100% successively[24]. Then coupons were examined using scanning electron microscope (SEM) (JEOL: JXA-840-Electron probe microanalyzer, Japan).

The biofilm formed on the metal surface was carefully removed and dried, mixed thoroughly with potassium bromide (KBr) and made as pellets according to Rajasekar[25]. These pellets are subjected to FTIR spectra (JASCO FT-IR 6100, Japan) to find out the biofilm formed on the surface of the metal coupons. FTIR spectrum was recorded in the mid region of 4000 cm^{-1} and 400 cm^{-1} at a spectral resolution of 4 cm^{-1}.

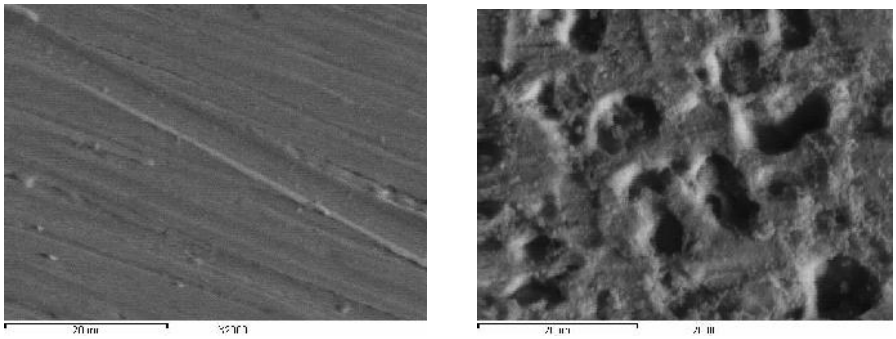
Polarization study:

The electrochemical measurements were carried out in a glass electrochemical –cell equipped with three electrodes, at 30°C temperature. Mild steel coupons (flag-shaped, 1 x 1 x 0.2 mm) were used as working electrode (WE). A platinum plate and a saturated calomel electrode (SCE) were used as a counter electrode (CE) and a reference electrode (RE), respectively[26]. The Tafel polarization curves were obtained according to Anandkumar[27] using (Potentiostat/ Galvanistat EG/G273). The electrochemical measurements were carried out with potential range between (± 250 mV vs. SCE) and scan rate of 0.5 mV/s.

RESULTS:

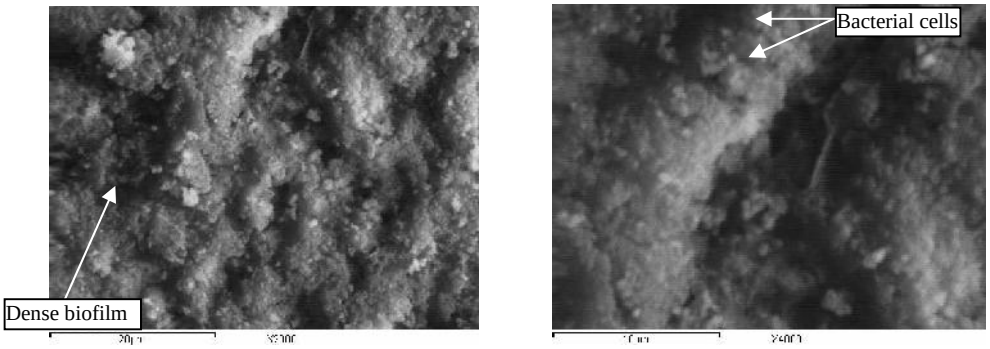
The surface examination of the blank using SEM (figure: 2a) represented smooth and shiny mild steel coupon surface while the control (figure: 2b) showed that roughness and some inclusions of corrosion products covered the whole coupon surface, while coupon surface exposed to *Pseudomonas marginalis* (figure: 3) was covered with intense biofilm, bacterial cells were impeded in it and corrosion products were clearly visible. The coupon surface exposed to *Desulfomonas pigra* (figure: 4) was not completely covered with biofilm, although, clusters of black deposits, corrosion products, biofilm layer contained bacterial cells and pits were obviously present. The coupon surface exposed to the mixed culture of *P. marginalis* and *D. pigra* (figure: 5) showed heavy accumulation of corrosion products, dense biofilm layers and bacterial cells.

Scanning electron microscopy examination of the coupon surfaces:



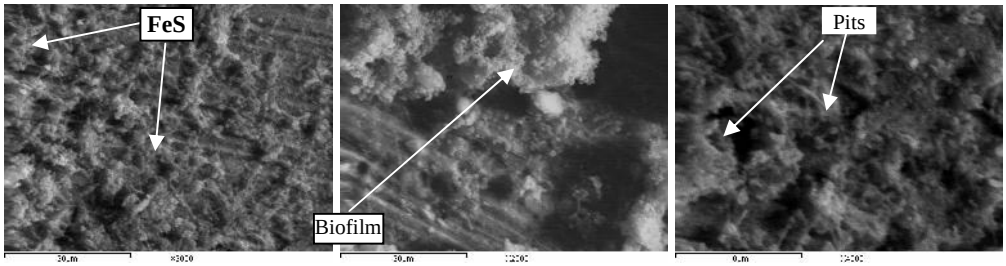
(a) **(b)**

Figure (2): Scanning electron microscopy (SEM) image of mild steel surface [X 2000]; (a): immediately after grinding and degreasing (Blank) and (b): exposed to sterile medium for 21 days at 30°C (Control).



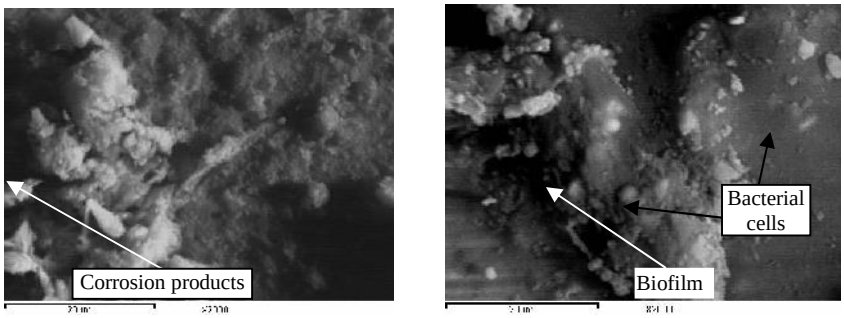
(a) (b)

Figure (3): SEM of mild steel coupon surface exposed to sterile medium inoculated with ***P. marginalis*** under static condition for 21 days at 30°C; (a): [X 2000] and (b): [X 4000].



(a) (b) (c)

Figure (4): SEM of mild steel coupon surface exposed to sterile medium inoculated with both ***D. pigra*** under static condition for 21 days at 30°C; (a) and (b): [X 2000] and (c): [X 4000].



(a) (b)

Figure (5): SEM of mild steel coupon surface exposed to sterile medium inoculated with ***P. marginalis*** and ***D. pigra*** under static condition for 21 days at 30°C; (a) and (b): [X 2000].

FT-IR spectroscopic analysis of the biofilm formed on mild steel coupon surfaces:

The FT-IR spectra of the biofilm formed on the surface of mild steel in the presence of *P. marginalis*, *D. pigra* and in mixture of both organisms figures (6, 7 and 8) showed the presence of polysaccharides and proteins which characterize a standard biofilm. Polysaccharide was characterized by many bands specifically a band which is a region of overlap corresponds to C–O–C and C–O–H stretching vibration[28]. Protein was also characterized by many bands specially the bands which represent amide I and amide II bands ascribed to peptide bond of polypeptides (proteins)[29]. These results combined with SEM showed that both *P. marginalis* and *D. pigra* had the ability to form biofilm on the surface of mild steel under investigation. *P. marginalis* had a greater ability to form a dense biofilm compared with that in case of *D. pigra*.

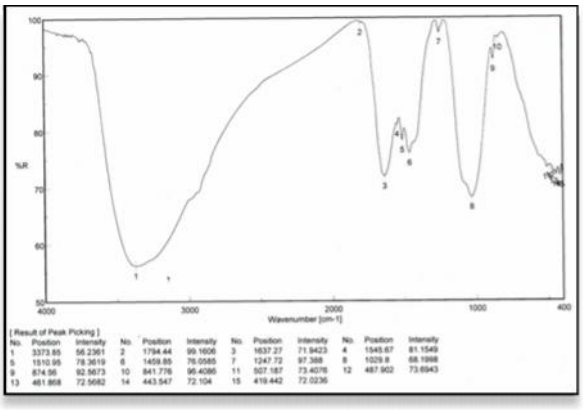


Figure (6): FT-IR of the biofilm formed by *P. marginalis* on the surface of metal coupon.

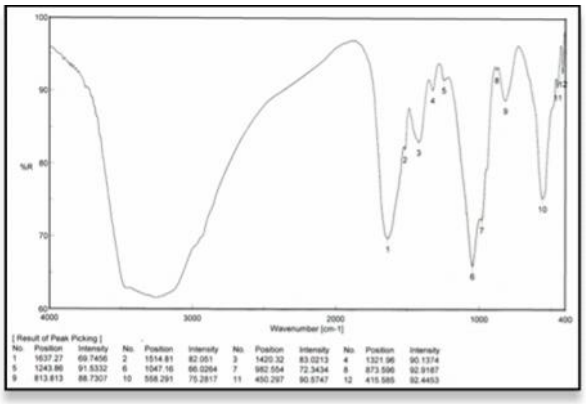


Figure (7): FT-IR of the biofilm formed by *D. pigra* on the surface of metal coupon.

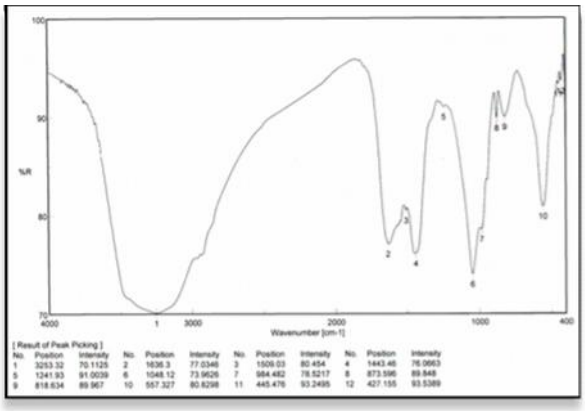


Figure (8): FT-IR of the biofilm formed by *P. marginalis* and *D. pigra* on the surface of metal coupon.

Electrochemical measurement:

Table (1) and figure (9) shows the Tafel polarization curve and corrosion behavior of the mild steel under investigation immediately after grinding and degreasing (Blank), exposed to sterile GYM medium (Control), *P. marginalis*, *D. pigra* and *mixture* of *P. marginalis* and *D. pigra* for 21 days at 30°C. Data showed that mild steel at zero time (blank) exhibit an active anodic dissolution with E_{corr} of (-628.9 mV), polarization resistance (Rp) of (0.302 KOhms) and corrosion rate of (15.5 mpy). In case of the mild steel exposed to sterile GYM medium (Control), there are an obvious shift of the corrosion potential to less negative value (-460.9 mV) indicating less susceptibility to corrosion process, increase of polarization resistance (Rp) to (0.839 KOhms) and decrease of corrosion rate to (9.5 mpy) due to the formation of a protective

passive film on the coupon surface. When the mild steel exposed to *P. marginalis* or *D. pigra*, the polarization curve shifted to cathodic Tafel region (-576.0 and -575.0 mV, respectively) with a greater susceptibility to corrosion process compared with control. *P. marginalis* reduced the polarization resistance (Rp) to about minimum (0.356 KOhms) compared with blank, while corrosion rate increased to maximum (25.1 mpy) indicating removal of the passive layer. *D. pigra* reduced the polarization resistance (Rp) to (0.538 KOhms) and the corrosion rate increased to (11.1 mpy). The mixture of *P. marginalis* and *D. pigra* stimulated the corrosion process as they caused a high increase in the corrosion potential (-681.5). In spite of the high increase in polarization resistance (Rp) to (1.362 KOhms) and decrease in corrosion rate to (4.5 mpy), it may be alert for a localized type of corrosion.

Table (1): Electrochemical polarization parameters:

Media	Linear Polarization				Tafel Polarization		
	$E_{Corr.}$, mV	$I_{Corr.}$, «A/cm^2	Corrosion rate, mpy	Rp KOhms	$E_{Corr.}$, mV	$I_{Corr.}$, «A/cm^2	Corrosion rate, mpy
Blank	**-628.9**	**34.2**	**15.4**	**0.302**	**-628.9**	**34.2**	**15.5**
Control	**-460.9**	**12.3**	**5.6**	**0.839**	**-460.9**	**20.9**	**9.5**
P. marginalis	**-576.0**	**29.0**	**13.2**	**0.356**	**-576.0**	**55.3**	**25.1**
D. pigra	**-538.2**	**19.2**	**8.7**	**0.538**	**-575.0**	**24.5**	**11.1**
Mixture of *P. marginalis* and *D. pigra*	**-681.5**	**7.6**	**3.5**	**1.362**	**-681.5**	**10.0**	**4.5**

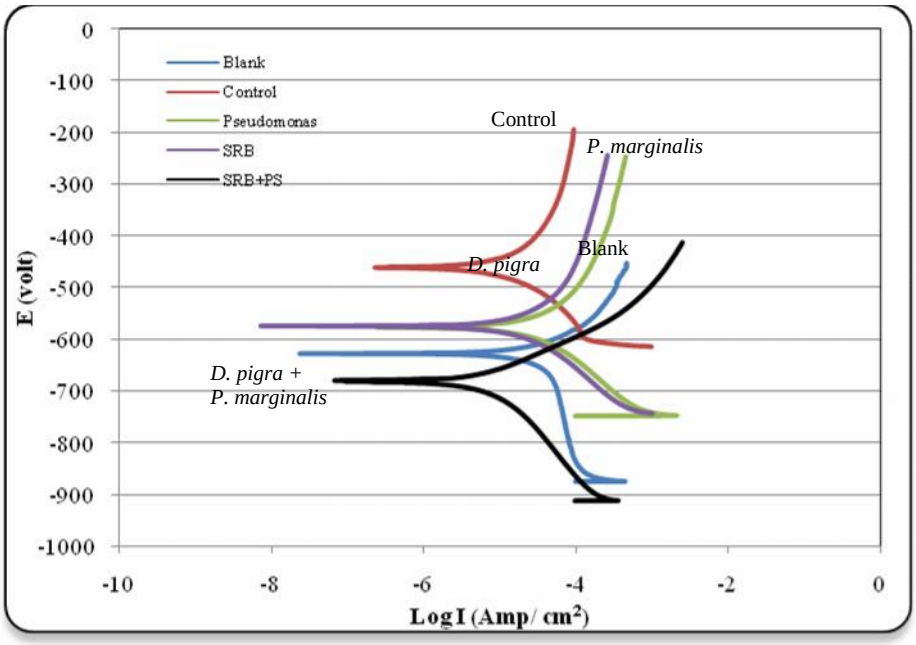


Figure (9): Corrosion behavior of mild steel using Tafel polarization curves of mild steel; at zero time (Blank), non inoculate at the end of incubation (Control), in presence of *P. marginalis*, *D. pigra* and in mixture of them. Media were incubated for 21 days at 30°C.

DISCUSSION:

Studying of biocorrosion using chemical, electrochemical and biological investigations have been evaluated by many workers [6, 15]. Effect of *P. marginalis* and *D. pigra* in single and in mixed culture was evaluated in this work using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and electrochemically (Tafel polarization curve). FTIR analysis of the sticky material on the coupon surfaces showed that *P. marginalis* and *D. pigra* were able to form typical biofilms. These results can be supported by Beech and Gaylarde[30] who verified that bacteria of the *Pseudomonas* species are usually related to cases of biocorrosion and are potential producers of exopolysaccharides that promote the adherence of the *Pseudomonas* and other microorganisms, resulting in the subsequent colonization of the metallic surface. Castaneda and Benetton[31] stated that localized damage of metallic surface was enhanced by SRB biofilm formation and metabolism. The electrochemical results and scanning electron microscopy images showed that the surface of the control coupon was completely covered by abiotic corrosion products (general corrosion) that acted as a protective passive film of the metal causing reduction in the anodic dissolution. In the potentiodynamic curve, the passive region was followed by rapid rise in the passive current (I_{pass}) at certain critical breakdown potential (E_b) indicating pitting corrosion. This localized type of corrosion was mainly due to the presence of the aggressive ingredients and penetration especially at the flaws and defects in the passive film under the influence of electrostatic field when these ingredients ions reached to the metal surface. The promoted local anodic dissolution resulting in a pit initiation, growth and propagation took place rapidly.

Data revealed that *P. marginalis* stimulated corrosion of the mild steel via anodic dissolution of metal. *P. marginalis* as shown in SEM can form intense biofilm on the surface of mild steel. The mechanism of corrosion enhancement by *P. marginalis* can be explained according to Videla and Herrera[32], who facilitating the removal of protective films when the biofilm detaches or through altering the structure of inorganic passive layers and increasing their dissolution and removal from the metal surface and changing oxidation–reduction conditions at the metal–solution interface by drastically changing the types and concentrations of ions, pH, and oxygen levels (the formation of differential aeration and concentration cells) and formation of corrosive products (e.g. H_2O_2 and acid metabolites) [33, 34, 35, 36, 37].

On the other hand, the presence of *D. pigra* decreased the protectiveness of the passive film (Abiotic one) and enhanced the susceptibility of the mild steel towards pitting corrosion. The mechanism of corrosion enhancement by *D. pigra* was illustrated and summarized by Videla and Herrera[32] and including indirect effect in which SRB produces metabolites (sulfides, hydrogen sulfides, thiosulfate, etc.) that are corrosive to mild steel and cathodic depolarization by SRB hydrogenase enzyme[38]. The presence of sulfide deposits also assist the occurrence of crevice or under deposit corrosion[39, 40] as it can be seen in figure (4c) indicating a more disastrous situation.

Nevertheless, in the presence of the mixed culture of *Pseudomonas marginalis* and *Desulfomonas pigra*, it was observed that a slimy film (BF) of different types of bacteria and biological secretion was formed and covered the mild steel surface during 21 days. Moreover black deposits of FeS as corrosion product were observed on the sample surface (figure: 5). The biofilm formed by *P. marginalis* and *D. pigra* was so sticky and protective to the mild steel surface, resulting in reduced corrosion rate. These conditions may induce localized corrosion[41] rather than protection of the mild steel. This was indicated by corrosion potential which was shifted to more negative value.

In contrast to the obtained results, Xu[42] showed that the combination of sulphate-reducing bacteria (SRB) *Desulfovibrio sp.* and iron oxidizing bacteria (IOB) of the *Leptothrix sp.*, which were isolated from the system for cooling water in the refinery, induce a higher degree of corrosion of SS316L than each of mentioned bacteria species separately.

CONCLUSION:

MIC is rarely linked to a single mechanism or to a single species of microorganisms. Biofilm forming bacteria and SRB may mediate interactions between metal surfaces and the liquid environment, leading to major modifications of the metal–solution interface. As a consequence of these changes, the electrochemical behavior of the metal can be modified from active to passive and even a microbial inhibition of corrosion can be reached. Despite of the increase of the corrosion rate stimulated by *P. marginalis* or *D. pigra* in single cultures, the mixed culture reduced the corrosion rate compared with control. The mutual effect of mixed culture can be completely different from the single cultures on the corrosion behavior of mild steel. The formation of sticky biofilm on the surface of mild steel may reduce general corrosion and may induce localized corrosion. The use of new

analytical tools, molecular biology methods, and innovative electrochemical devices facilitate biocorrosion investigation is recommended. There is no ideal model of MIC system that can be applied to all cases, but MIC study is a case-specific and had so many variables.

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