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CORROSION OF MILD STEEL EXPOSED TO NATURAL TAPE-WATER BY *PSEUDOMONAS DIMINUTA*

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ABSTRACT :

Pseudomonas diminuta was isolated from iron based deposits (rust layer) on nutrient agar medium. For corrosion's determination of mild steel (MS) two different techniques were used for Electrochemical Impedance Spectroscopy[EIS] expressed by (impedance [z, ohm], phase angle [degree]) and tafel polarization by using three test solutions (natural tape-water system [NTS], natural tape water containing iron-based deposits [NFS] and natural tape water containing *Ps. diminuta* and MS [NBS]). Parallel to the above experiment, Colony Forming Units [CFUs/ml] in absence or presence of MS was determined (0-10 days). The results of impedance showed a decrease followed by fixed values and a vice versa results were obtained in case of phase angles (°) shift at high and low frequency respectively. Tafel polarization expressed by circuit potential (E) mV were reduced in the presence of bacteria compared to its absence) in anodic current density [uA/cm^2]. All data obtained were done after one and four days of incubation in the above three media, also CFU increased after one day in presence of MS. It can be concluded, that the above bacterial species contribute to the corrosion of MS.

INTRODUCTION:

The physical presence of microbial cells on the surface, in addition to their metabolic activities modifies electrochemical processes[1]. Adsorbed cells grow, reproduce and form colonies that are physical anomalies on a metal surface[1]. Under aerobic conditions, areas under respiring colonies become anodic and surrounding areas become cathodic[2]. Any geometrical factor that results in a high oxygen concentration in one area and a low concentration in another will create a differential cell[2]. A mature biofilm prevents the diffusion of oxygen to cathodic sites and the diffusion of aggressive anions, such as chloride, to anodic sites. Outward diffusion of metabolites and corrosion products is also impeded. If areas within the biofilm become anaerobic, i.e. if the aerobic respiration rate within the biofilm is greater than the oxygen diffusion rate, the cathodic mechanism changes[1, 2].

Microbes perform oxidation and reduction reactions that profoundly affect the stability of minerals in the environment, with consequences

ranging from the promotion of acid mine drainage[3] to the bioremediation of organically polluted groundwater[4].

In industrial settings, perhaps the most familiar metal transformation is the rusting of iron, steel, and microbes are thought to play an important role in this process[5].

Microbiologically influenced corrosion (MIC) can be a serious industrial problem and affects diverse processes ranging from water distribution in cast iron mains and sewers to transport of natural gas in steel pipelines. It has been estimated that for the United States oil industry alone, MIC causes hundreds of millions of dollars in damage to the production, transport, and storage of oil every year[6]. The goal of the present work was concerned with the isolation and identification of the most popular bacteria population on the corrosive iron deposits and investigation of its survival times in the presence of MS which can help in the occurrence and survival of the predominant bacteria strain in biofilms and pipeline system.

EXPERIMENTAL APPROACH:

1-Bacterial isolation:

The bacterial strain was isolated from corrosive iron deposits sample gathered from Microbial taxonomy and physiology laboratory, Botany and Microbiology Department, Faculty of Science, Al- Azhar University-Assiut. and identified in the same lab. Corrosive iron deposits was added at the level of 0.1% (w/v) and applied using serial dilutions technique using peptone water medium.

2-Bacterial purification :

The purification procedure of the bacterial isolate under investigation was carried out by the agar streak plate method. All colonies of different forms and color showing separate growth on the nutrient agar medium were picked up and restreaked following the zig-zag method onto the agar surface of plates containing the same isolation medium. At the end of incubation period, only the growth which appeared as a single separate colony of distinct shape and color was picked up and restreaked again for several consecutive times onto the surface of agar plate of the isolation medium to ensure its purity which was checked up microscopically and morphologically using Gram's stain. Pure isolates only were subcultured on slants of its specific isolation medium and kept for further investigation.

3-Bacteria identification:

The identification process has been carried out by the aid of Bergey's Manual of Systematic Bacteriology[7] (Volume 1), section 4, this isolate is suggestive of being belonging to *Pseudomonas* thus, it could be given the tentative name *Pseudomonas.* Also the characterization of this isolate was confirmed by Bergey's Manual of Determinative Bacteriology[18].

4-Total count of *Ps. diminuta*:

This was carried out on the presence and absence of Mild steel (which represent the working electrode and provided in sheets form) which used in direct exposure to the bacterial culture. At each survival time tested 1 ml was diluted as serial dilution technique and 0.1 ml of each dilution interval was distributed on the surface of Nutrient agar medium (Beef extract, 3.0 g; Peptone, 5.0 g; Sodium chloride, 3 g and Agar, 15 g; distilled water up to 1000 ml, dissolve by heating, adjust pH at 6.8. Sterilize at 121°C for 15 min.). The plates were incubated for 48h at 37°C. Then all colonies from the

dishes were counted by what so called Colony forming units (CFUs).

5-*Ps. diminuta* survival times:

Bacterial strain was counted on agar medium at different time (0, 1, 2, 4, 6, 8 and 10 days). At the end of each incubation interval, CFU techniques have been performed. Different serial dilutions of both bacterial isolates have been performed at 10^{-3} dilution. After each survival times, CFU techniques was counted in the presence or absence of MS samples.

6- Methods:

a-Materials:

Electrodes were prepared from mild steel (MS) 1018 (UNS G10118). The electrodes were provided in sheets form. Prior to each test the exposed surfaces were polished with SiC paper up to 1200 grid, and rinsed with distilled water.

b-Test solutions:

In order to study the effect of *Ps. diminuta* count ($39X10^3$), 3 sets of the test solution system have been used, natural tape-water system (NTS) (pH=7.5), natural tape water containing 0.1% (w/v) iron-based deposit (NFS) and natural tape water containing the previous count of from *Pseudomonas diminuta* (NBS).

c-Electrochemical Cell[9]:

The electrochemical cell conventionally consists of three electrodes, which are immersed in the test solution (Fig.1). The working electrode is the corroding metal which is mild steel with exposed area of 1 cm^2. The counter electrode is made from platinum.

The counter electrode is connected to a potential control device called a potentiostat. The potentiostat applies to the counter electrode whatever voltage and current are necessary to maintain the potential that is desired between the working and reference electrodes. The reference electrode is constructed so that it has a negligible contact potential regardless of the environment in which it is placed. The reference electrode is connected to a potentiometer. Saturated calomel electrode (SCE) has been used in this study as a reference electrode. All the parts of electrochemical cell were sterilized at 120 ^{0}C for 35 min. Two different techniques were used to monitor the corrosion behavior of mild steel exposed to tape-water with and without bacterial culture.

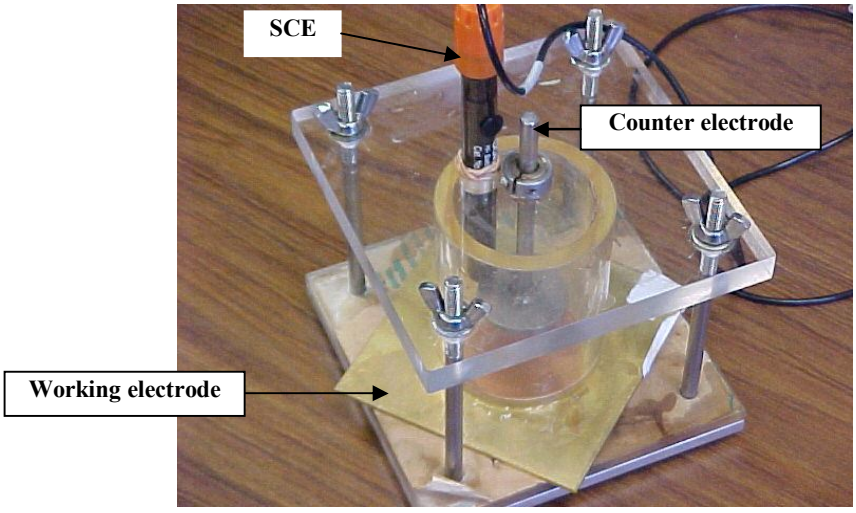


Fig. (1): Electrochemical cell

d-Electrochemical impedance Spectroscopy (EIS) and Phase angle [8-10]:

Impedance and Phase angle measurements were made with a model AUTO AC DSP device (ACM Instruments). A sinusoidal voltage signal of 10 mV was applied in a frequency range of 10^5 to 10^{-3} Hz.

e-Tafel polarization[10]:

Tafel polarizations were obtained using EG&G 273A potentiostat /Glvanostat system. The experiments were run directly after EIS measurements using the same cell used in EIS. The working electrodes were polarized -250 mV below the open circuit potential (E_{corr}) up to 250 mV above E_{corr} with scan rate of 0.2 mV s^{-1}. All EIS and Tafel measurements were conducted at E_{corr} at room temperature.

RESULTS AND DISCUSSION:

Isolation and Identification process were carried out on the most popular bacterial population of the iron deposits which represented in one strain was identified as *Pseudomonas diminuta* this was depending on its morphological, physiological and biochemical properties[7] and shown in Table (1).

The survival of *Pseudomonas diminuta* was monitored by its cultivability and count. When the samples were supplemented with the MS sample cell count of the bacteria was (39X103/ml) at zero time. Results confirmed that the counts were higher in the presence of MS samples compared to its absence at all survival times tested as in Table (2). Colony forming units ranged from the 223, 147, 107, 103, 89 and 50X103 compared with 88, 56, 55, 49, 44 and 32 X103 CFU ml-1 at survival times 2, 4, 6, 8 and 10 days, respectively. Table (2) shows the survival of bacterial isolate at different incubation times in the presence and absence of MS samples.

Table (1)

Character	
Cell shape	Bacilli
Gram reaction	-
KoH (3%) reaction	+
Motility	-
Spore formation	-
Oxidase	+
Catalase	+
Anarobiosis	Aerobic
Carbohydrate, acid from:	
L(+) Arabinose	-
Lactose	-
D(-) Fructose	-
D(-) Galactose	-
Glucose	-
Trehalose	-
D(-) Mannitol	+AG
D(-) Mannose	-
Raffinose	-
D(-) Ribose	-
L(-) Rhamnose	-
Sucrose	-
NaCl 2%	+
4-6%	+
8%	-+
10%	-
Hydrogen sulfide production	-
Nitrate reduction	Partial
Methyl red (M.R.) test	-
Voges proskauer (V.P.) test	-
Enzyme production:	
Amylase	+
Lipase	+
Cellulase	+
Aesculin	-
Urease	-
O/F	-/+
Indole	-
Pectinase	+

(S.F.): Spore former. (+): Positive. (-): Negative. (+WA): Positive weak acid. (±A): Doubtful acid (±): Doubtful. (+A): Positive acid. (+M): Motile (P.red): partial reduction. (Non-M): Non motile Facult: Facultitvely anaerobic.

Table (2)

T* (days)	CFU x10^3/ml in	
	absence of MS**	presence of MS
0	39	39
1	88	223
2	56	147
4	55	107
6	49	103

8	44	89
10	32	50

***T: time** ****MS: Mild steel**

Impedance spectra for MS samples obtained after 1 and 4 days of exposure to sterile natural tap-water system (NTS), inoculated natural tape water containing 0.1% (w/v) iron-based deposit (NFS) and natural tape water system containing 0.1 ml from *Pseudomonas diminuta* (NBS) media are illustrated in Figure (2).

When the frequency of the applied signal was very low ($f \rightarrow 0$, left side of the spectrum), impedance recordings reflected the *Rp* at the surface of the test electrode (in this case, 1018 steel). When the frequency was very high (right side of the spectrum), impedance recordings reflected the electrolyte resistance in solution. Rp is inversely proportional to the corrosion rate. As shown in Fig. (2) lower values of impedance were observed for NBS and NFS at all studied frequency range (10^{-2} to $3x10^4$ Hz.). At very low frequency, where the impedance will be considered as Rp, Impedance values were reduced to about 750 ohm in the presence of bacteria compared to about 1800 ohm in its absence. These results indicate that corrosion rate was increased in the presence of bacterial culture. The impedance values were almost unchanged in the high frequency parts (from about 10^{-2} to $3x10^4$ Hz.) for all systems and these values only refer to the solution resistance of the studied systems (Fig. 2).

Also Fig. (3) shows the impedance spectra for MS after 4 days of exposure. Impedance values at low frequency (10^{-2} Hz.) were also decreased to about 750 ohm for NBS to about 130, this followed by lowering of impedance value's range between 130-110 ohm at frequency's range 10-$3x10^4$ Hz in the presence of bacteria (Fig. 3). When the results of Fig. (2) are compared with those of Fig. (3) it can be concluded that for all studied systems, impedance values obtained after 4 days of immersion (Fig. 3) were lower than those obtained after 1 day (Fig. 2) which was expected due to the formation of non protective rust layer over the entire surface of MS.

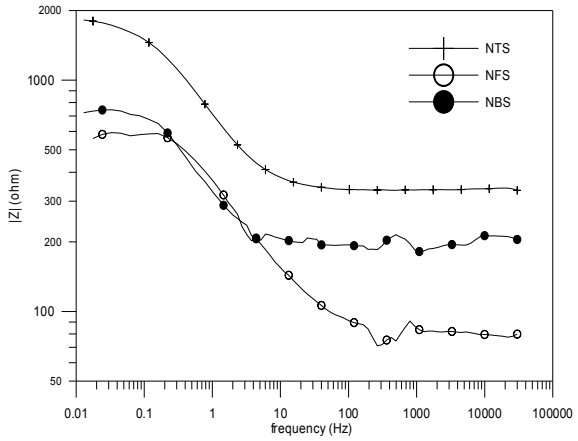


Fig. (2)

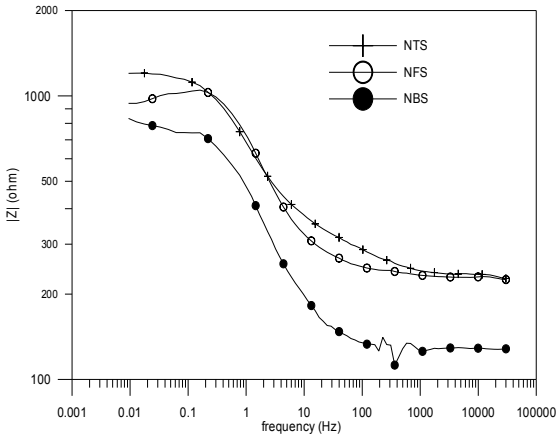


Fig. (3)

Impedance spectra for MS samples after one day (Fig. 2) and four days of exposure (Fig. 3)

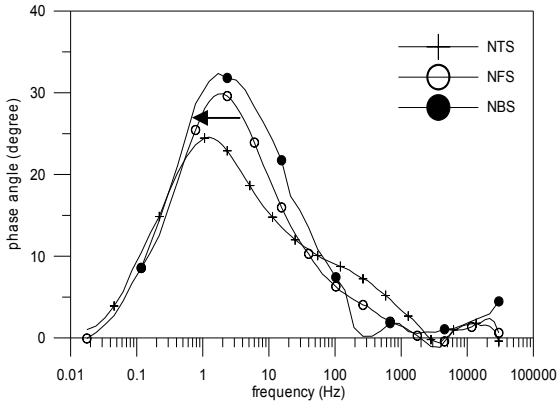


Fig. (4)

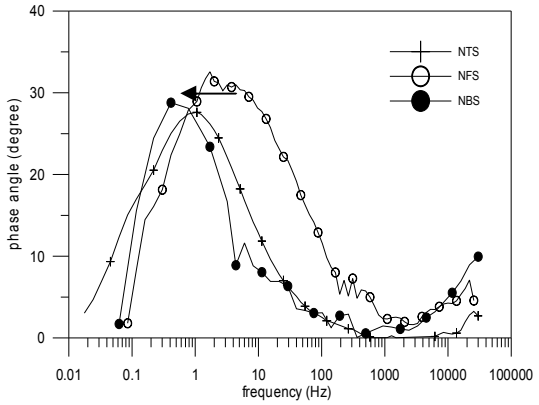


Fig. (5)

Phase angle diagram for MS samples after one day (Fig. 4) and 4 days exposures (Fig. 5)

Figs.(4) and(5) show a comparison between the phase shift observed for MS exposed to NTS, NFS and NBS after 1 and 4 days of exposure. A lot of useful information, such as the double layer capacitance, number of films covering the surface and the effect of diffusion could be obtained from studying phase shift diagrams[8-10]. The phase angle vs., frequency (f) plot in Fig. (4) showed maximum phase angles (where, phase angle (^{o}) was at 33 at the low frequency about (3 HZ) respectively, this followed by falling of phase angle (^{o}) range between 20-2 then shown a little increase to about 4 at high frequency's range of about $3x10^4$Hz (this little increase is believed to be due to interference of reference electrode). The results revealed that the interface response contains at one time constant and that a characteristic frequency, where the phase shifts shows a maximum, shifted in the low frequency side as the corrosion progressed (as indicated by the arrow in Fig. (4). Fig. (5) also shows only one time constant where, phase angle was at 28^o at the low frequency of about 0.4 Hz. Also the phase angle showed a maximum shifted in the low frequency side as the corrosion progressed. This shift indicates an increase in values of capacitance which is correlated to the observed decrease in impedance shown in both Figs. (2) and (3). The presence of one time constant in both Figs. (4) and (5) indicate that the corrosion mechanism is independent on time. In general, the presence of one time constant may indicate the presence of only one layer over the metal surface. This could be easily described by the simplest one time constant model [8-10].

To obtain Rp values from the previous impedance spectra (Figs. 2 and 3) the impedance spectra for MS was analyzed using the equivalent circuit (EC) shown in Fig. (6). The elements used in this EC are CPE, R_s, R_p. Where, CPE is the constant phase element, Rp and Rs is the solution resistance.

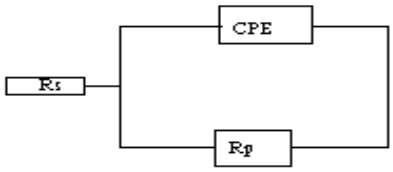


Fig.(6): Equivalent Circuit (One time constant model)

In studies of corroding systems, many authors use CPE to model surface heterogeneity caused by many factors such as: adsorption of inhibitors, impurities, surface roughness, etc.[11-13]. In other words, CPE is most often used to describe the frequency dependence for the

non-ideal capacitive behavior. The impedance of CPE elements is given by[14]:

$$Z_{CPE} = [Q (j \omega)^n]^{-1} \quad \text{.....(1)}$$

Where j is the imaginary number, Q is the frequency-independent real constant, $\omega = 2\pi f$ is the angular frequency (rad/sec), f is the frequency of the applied signal and n is the CPE exponent which is also known as roughness factor or surface inhomogeneity[11-15].

Fig. (7) shows the experimental and fitted impedance data for MS exposed to tape-water for 1 day. EIS data fitting were performed using Boukamp's software (EQUIVCRT)[16]. Excellent agreement between the fitted and experimental data was obtained indicating that the EC circuit shown in Fig. 6 (one time constant model) is the best for describing the corrosion system in this study.

In general, higher Rp values indicate a good corrosion resistance whereas smaller values indicate a poor corrosion resistance. Fig.(8) shows a comparison between Rp values obtained for MS exposed to NTS, NFS and NBS media. It is clear that smaller Rp values were obtained in the presence of *Pseudomonas diminuta* (NBS medium) ($6.5x10^2$ ohm.cm^2) than that obtained for MS exposed NTS and NFS (from about $7x10^2$ to $1x10^3$ ohm.cm^2) which suggest that corrosion were accelerated in the presence of bacteria.

In general, Rp values obtained in this study decreased with time and remained almost unchanged after about 4 days of immersion (Fig. 8). These results are in consistence with those shown in table (2) where the maximum values of CFU were observed after about 4 days then remain almost constant.

Figs. (9 and 10) show Tafel polarization for MS exposed to NTS, NFS and NBS media for 1 and 4 days, respectively. Tafel polarization (log (*I*) versus *E* curves) allowed the determination of the electrochemical reactions involved in the corrosion process.

Fig. (9) shows the influence of *Pseudomonas diminuta* (NBS medium) on the anodic and cathodic polarization curves. The working electrodes were polarized -250 mV below the open circuit potential (E_{corr}) up to 250 mV above E_{corr} with scan rate of 0.2 mV s^{-1}. E_{corr} where found to be near -700 mV. Accordingly, the lower part of the curve (from about -950 to about -700 mV) represents the cathodic curves whereas the upper part (from about -700 in the presence of bacterial isolate to-450 mV in its absence) corresponding to the anodic curves. An increase on the anodic current density was observed in the presence of *Pseudomonas diminuta.* The arrow shown in the upper part of Fig.(9) clearly shows how the anodic current density increased in the presence of bacteria. The anodic current density was increased from about $1x10^{-3}$ uA/cm^2 to about $3x10^{-3}$ uA/cm^2 in the presence of *Pseudomonas diminuta.* The cathodic current curves were almost identical regardless the presence or absence of bacterial population. The almost identical cathodic current density curves indicating that the corrosion mechanism is not under cathodic control[9]. Similar results were obtained for MS exposed to NTS, NFS and NBS media after 4 days (Fig. 10). The anodic current density increased to from about $2x10^{-3}$ uA/cm^2 to about $9x10^{-3}$ uA/cm^2 in the presence of bacteria. In general, for all the studied systems, lower anodic current values where observed in Fig. (10) than those obtained in Fig.(9) indicating acceleration of corrosion with time. Based on the previous findings, it can be concluded that Tafel polarization results successfully correlate the corrosion behavior of MS samples. Also, the previous results showed that both Tafel and EIS are in agreement with each other.

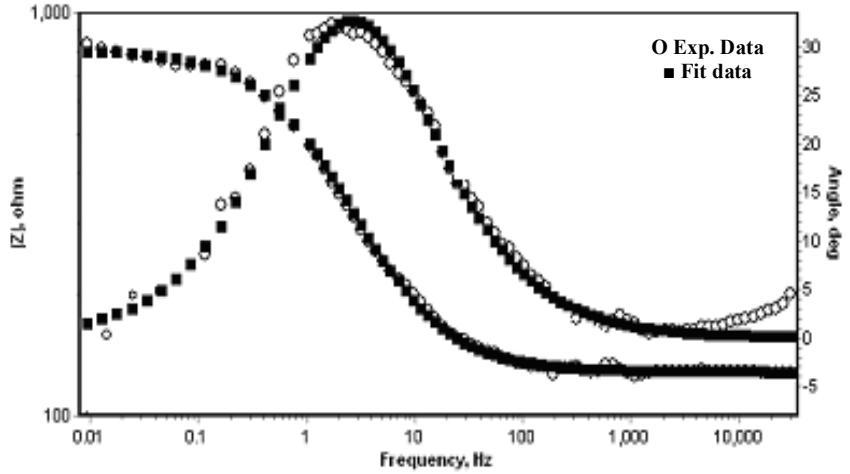


Fig.(7): Comparison of experimental and fit data for MS samples exposed to NTB medium for 1 day

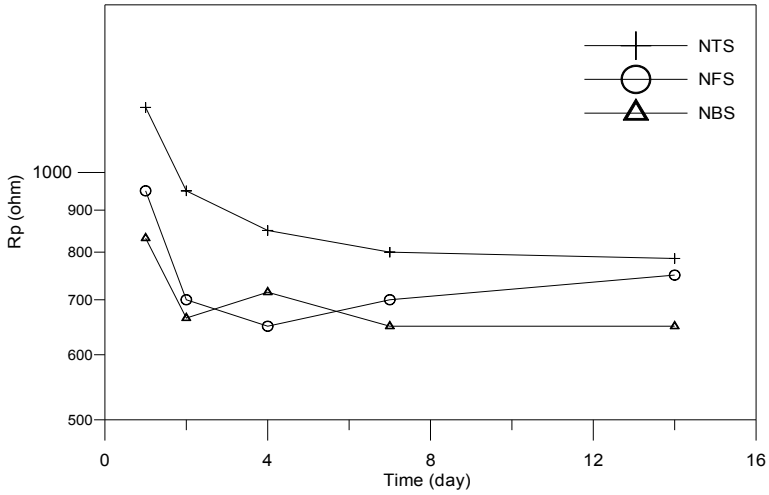


Fig. (8): Time dependence of Rp for MS exposed to different media

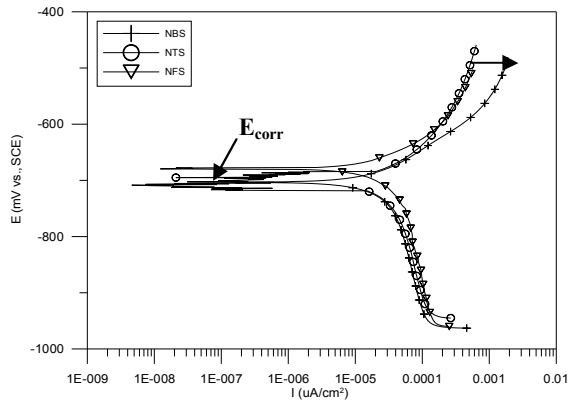


Fig. (9)

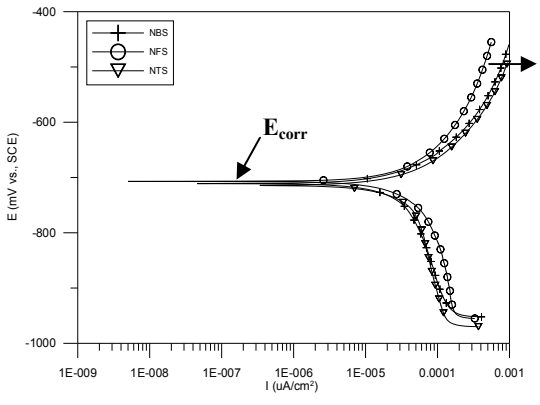


Fig. (10)

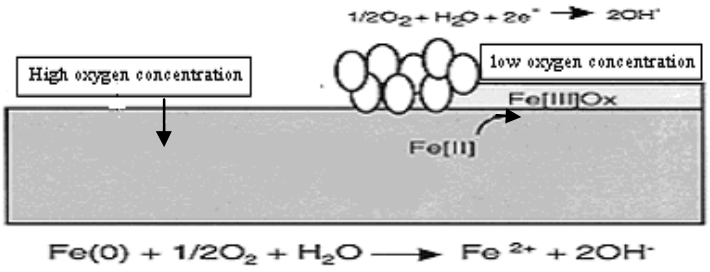
Tafel polarization curves for MS exposed to different media for one day (Fig. 9) and 4 day (Fig. 10)

The surfaces of MS samples have been examined under optical microscope after the exposure test. It was noticed that a patchy rusted area were found over the surface for samples exposed to NBS system whereas in the absence of *Pseudomonas diminuta* a uniform rust layer covered the entire surface of MS samples. This could be attributed to the formation of a patchy biofilm over MS samples. It was reported that both aerobic and anaerobic respiration can significantly contribute to the corrosion phenomena in systems in which water is quiescent.

Depending on the chemistry of the water in which iron oxidation occurs, in the presence of iron-reducing bacteria resulting corrosion product(s) may prevent oxygen attack by forming a physical barrier at the surface. If the corrosion product contains Fe(III), iron reducers may utilize this substrate as a terminal electron acceptor for anaerobic respiration, and the resulting Fe (II) may also scavenge oxygen in the water column leading to corrosion protection[17]. This concept will be true if the biofilm completely covered MS samples as was found before[8-10]. However, in this study a patchy biofilm was observed. Consequently, expected galvanic cells (differential aeration cells) will be formed over MS samples in the presence of *Pseudomonas diminuta* due to unequally oxygen concentrations over MS samples leading to acceleration of corrosion[1, 2]. Generally galvanic cell refers to a process in which chemical energy generated from spontaneous redox (anodic and cathodic reactions) reaction is changed to electrical energy. The following diagram shows how oxygen will be consumed by direct microbial oxygen respiration and indirect reduction by ferrous iron (produced by ferric iron respiration). The diagram also shows two areas one containing high oxygen concentration and one with low oxygen concentration.

The ovals represent cells that are actively respiring at the steel surface. Ferrous iron produced by respiration of Fe (III) oxides forms a reducing shield that blocks oxygen from attacking the steel surface. After the exposure tests the MS surface have been cleaned by nitric acid to remove the corrosion products and the exposed surface was examined under an optical microscope to determine if there is any pit found over MS samples. No pits were found indicating that uniform corrosion was the main corrosion mechanism in this study.

Our results indicate that biofilms comprising *Pseudomonas diminuta* may accelerate the corrosion rate of steel due to formation of a galvanic cell over the surface of MS samples. The results indicate that the damage observed on the metal surface depends upon the sessile microorganism's population. Due to this situation, it is very important to determine the sessile kinetics growth when studying MIC processes. Also the results showed the dramatically altered quantity of CFU in the presence and absence of MS samples. Further studies must be undertaken to delineate the exact mechanism by which this strain contribute to corrosion.



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تآكل الحديد المطاوع المعرض لمياه الصنبور الطبيعية بواسطة بكتيريا السيدوموناس ديمنيوتا

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اشتملت الدراسة على عزل وتعريف الفلورا البكتيرية من عينة الحديد المتآكل (طبقة الصدأ) على وسط الاجار المغذى. ولتحديد درجة التآكل تم استخدام طريقتين (سبكتروسكوبية المعاوقة الكهروكيميائية (EIS) ومخططات تافل Tafel polarization فى وجود ثلاثة محاليل (مياه الصنبور الطبيعية المعقمة (NTS) ومياه الصنبور المعقمة المحتوية على برادة الحديد (NFS) ومياه الصنبور المعقمة المحتوية على الحديد المطاوع Mild Steel (MS) و *Pseudomonas diminuta* (NBS) بالتوازى مع هذه الطريقتين تم استخدام طريقة العد البكتيري ((Colony Forming Units (CFUs/ml) فى وجود أو غياب الحديد المطاوع Mild Steel (MS) فى أوقات تحضين من (صفر إلى 10 أيام).

ومن دراسة سبكتروسكوبية المعاوقة الكهروكيميائية أمكن الحصول على قيم المعاوقة المستقطبة (Rp) والتى وجد أنها قلت ثم وصلت إلى معدلات ثابتة والعكس بالعكس أظهرت قيم الطور الزاوى (phase angle) عند الترددات المنخفضة والمرتفعة. أظهرت النتائج الخاصة بمخططات تافل أن قيم المخططات الأنودية المستقطبة (Anodic Polarization Curve) قد اتجهت إلى معدلات أقل في وجود البكتيريا بالمقارنة بغيابها. وكانت كل النتائج المتحصل عليها قد أجريت بعد اليوم الأول والرابع من التحضين فى وجود الثلاثة أوساط السابقة، وأيضاً اد معدل النمو البكتيرى بعد اليوم الأول من التحضين فى وجود الـ (MS). بناء على ذلك نستطيع القول بأن سبب التآكل على سطح المعدن الـ (MS) راجع إلى وجود البكتيريا المستخدمة مما سيكون له أبلغ الأثر في تطبيق هذه البكتيريا على المستوى التجريبي والمعملي ومعرفة مدى تطبيقها.