The Influence of AISI4100 Low Carbon Alloy Steel Corrosion by Sulphate-Reducing Bacteria Isolated from Pasir Gudang, Johor

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ABSTRACT

The influence of sulphate-reducing bacteria (SRB), *Desulfovibrio* sp. on the anaerobic corrosion of low-carbon alloy steel was evaluated. SRB were isolated from the vicinity of Malaysia Marine and Heavy Engineering (MHHE) Sdn. Bhd., Pasir Gudang, Johor and grown in VMNI medium. Potentiodynamic polarisations and Electrochemical Impedance Spectroscopy (EIS) were performed, both in inoculated and sterile medium. Tafel analysis of the polarisation curves around the corrosion potential, E_{corr}, showed the presence of SRB-induced changes in the corrosion rate in VMNI medium. EIS results showed that the diameter of Nyquist semicircle of alloy steel immersed in the control was greater than the VMNI containing *Desulfovibrio* sp. Scanning electron microscope observation of alloy steel coupons, exposed to the SRB action, revealed the presence of bacteria, as well as damaged steel surface. A type of localised corrosion was observed on the metal surface and it was associated with the SRB effect. Electrochemical measurements showed that the strong acceleration in the pitting corrosion process was induced by *Desulfovibrio* sp.

Keywords: Tafel analysis; electrochemical impedance spectroscopy (EIS); scanning electron microscopy (SEM)

Introduction

Sulphate-reducing bacteria (SRB) have been described as an anaerobic bacteria group, which undergoes dissimilatory sulphate reduction (Posgate, 1984). Since SRB show

considerable adaptability to extreme conditions, they are widespread in various ordinary environments. The growing interest in this bacteria group is connected with the indication of their role in the corrosion of metal constructions. Among several genera of SRB, the genus *Desulfivibrio* is the best known, because members of these genera can be relatively easily isolated and purified (Hamilton, 1985). Most strains of *Desulfovibrio* can reduce sulphate using gaseous hydrogen as an electron donor. It is emphasised in the literature concerning biocorrosion that no specific mechanisms of microbial-enhanced corrosion have yet been described.

Microbial influence corrosion, MIC, is undoubtedly a phenomenon of great importance (Hamilton, 1985; Lee et al., 1995). The anaerobic corrosion of alloy induced by the sulphate-reducing bacteria, SRB, is of particular interest, both economically and scientifically. The pitting corrosion of alloy steel in SRB media has been shown by many researchers (Videla, 1996; Raiha & Fonseca, 1996). The main difficulties in understanding this phenomenon arise from the complexity of the chemical composition of these mediums.

On metals, the formation of biofilms causes the ennobling of their free corrosion potentials to values where the initiation, propagation of pitting and crevice become feasible (Johnsen & Bardal, 1986; Dexter & Gao, 1988). In the original concept, as formulated by von Wolzogen Kuhr and van der Vlugt in 1934, SRB promoted biocorrosion of cast-iron metal surfaces anaerobically through cathodic depolarisation (Ehrlich, 1997). In this model, an iron surface exposed to aqueous moisture undergoes the spontaneous reaction, and the half-reaction are as follows:

$$2H^+ + 2e^- \longrightarrow 2H \qquad \text{(cathode)} \qquad (1)$$

$$2H_2S + 2e^- \longrightarrow 2HS^- + H_2 \qquad (2)$$

$$4H_2 + SO_4^{2-} \xrightarrow{\textit{anaerobic bacteria}} 4H_2O + S^{2-} \qquad \text{(cathodic depolarisation)} \qquad (3)$$

$$Fe^{2+} + S^{2-} \longrightarrow FeS \qquad \text{(anode)} \qquad (4)$$

overall reaction:

$$4Fe + SO_4^{2-} + 4H_2O \longrightarrow FeS + 3Fe(OH)_2 + 2OH^- \qquad (5)$$

The H_2 generated in the cathodic region was thought to accumulate at the iron surface where its build-up can cause passivation (polarisation) of the surface and thus, can stop further corrosion. In the case when SRB was present, H was removed from cathode by SRB metabolism activity, reducing SO_4^{2-} to S^{2-}. This phenomenon is known as cathodic depolarisation reaction. The sulphide generated could react with the Fe^{2+} produced at anodic areas, which would promote corrosion if iron sulphide did not precipitate on the iron surface as a uniform film that would increase the passivity of the iron surface as long as the film was undisturbed (Ehrlich, 1997).

Electrochemical impedance spectroscopy (EIS) is a powerful method to study the surface condition of metals because some of the time constants involved in the surface

structure can be discriminated. EIS was used to investigate the electrochemical behaviours and to measure the polarisation resistance of the alloy steel (Mansfeld & Lorenz, 1991). Kinetic and thermodynamic characteristic of the corrosion process will be observed by Tafel analysis of the polarisation curves (Keresztes et al., 1997; Rainha & Fonseca, 1997). According to Dexter et al. (1991) and Hamilton & Lee (1995), anodic and cathodic curves shift are of most importance to the study of corrosion processes caused by microbials.

This paper reports the changes of electrochemical parameters for the corrosion of alloy steel by SRB activities grown in VMNI medium. The present study is designed to gain a better understanding of SRB influence on the stability of alloy steel using electrochemical impedance spectroscopy (EIS), Tafel analysis of the polarisation curves and scanning electron microscopy (SEM) techniques. With this technique, it is possible to measure extremely low corrosion rates and can be used for continuing monitoring of the corrosion rate of the system.

Materials and Methods

Cultural Conditions

The *Desulfovibrio* sp. used in this work was taken from the Malaysia Marine and Heavy Engineering Sdn. Bhd., Pasir Gudang, Johor. The collected samples were inoculated in a selective medium, following the recommendations for SRB sampling. The microorganisms were maintained in the laboratory using the VMNI medium (Table 1) proposed by Zinkevich et al. (1996) which was modified from Posgate's Marine medium C. The medium was degassed under N_2 for 30 minutes to create anaerobic condition and the pH was adjusted to 7.2 using 1.0M NaOH before autoclaving at 121°C. It was left to cool to room temperature before being inoculated with the SRB.

TABLE 1: Composition of the VMNI Medium

Chemical Reagents	Composition (g/L)
KH_2PO_4	0.5
NH_4Cl	1.0
$NaSO_4$	4.5
Sodium citrate	0.3
$CaCl_2.6H_2O$	0.04
$MgSO_4.7H_2O$	0.06
Casamino acids	2.0
Tryptone	2.0
Lactate	6.0
Ascorbic acids	0.1
Thioglycollic acid	0.1
$FeSO_4.7H_2O$	0.5
Trace elements (stock solution)	1.0 ml
Vitamins (stock solution)	2.0 ml

The bacterial cells were spun in 30 ml centrifuge tubes for 10 minutes at 1200 rpm, the supernatant was removed and the samples were ready to be used or stored in the freezer until needed.

Electrochemical Experiments

These experiments were carried out according to ASTM standard cell (ASTM Designation G3-89 1999), with a three electrode system: low carbon alloy steel as working electrode, a graphite rod as counter electrode and a saturated calomel electrode as reference electrode (Figure 1). The electrolyte used was 300 ml VMNI medium. Nitrogen gas was bubbled to remove all the oxygen and maintain anaerobic conditions. The carbon alloy steel samples (as a working electrode) were immersed in the electrolyte solution exposing a circular area of about 0.708 cm^2. All the experiments were performed using Autolab PGSTAT30. The electrochemical cell was connected to an Autolab PGSTAT30 and PC was used for data recording. All experiments were performed at 35 C and during this time, several polarization resistance tests were carried out.

A potentiodynamic method was used to obtain the potential-current ratio, applying ± 10 mV over potential, with respect to the free corrosion potential, E_{corr}. The 'General purpose Electrochemical System' (GPES) software program was used for data management and analysis.

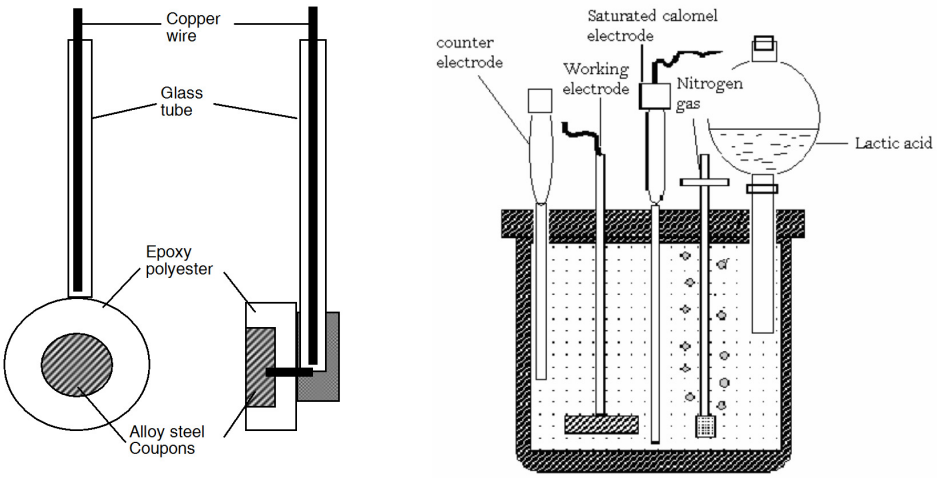


FIGURE 1: (a) Schematic Diagram of Alloy Steel Concentric Electrode (As a Working Electrode) and (b) Sketch of the Electrochemical Cell

From this technique, resistance values were obtained (R_p), which were used to calculate the corrosion current density, according to the following equation:

$$I_{corr} = \frac{B}{R_p}$$

where B is the Tafel slope Faradays's law which can be used to relate the corrosion current density to the corrosion rate, with the next equation:

$$\text{Corrosion rate (CR)} = \frac{K \times I_{corr} \times EW}{D}$$

where K is constant = 3272, EW is equivalent weight and D is density.

The impedance measurements were carried out on a computer-interfaced Autolab PGSTAT 30 equipment (Eco. Chemie B. V., Netherlands) together with frequency response analysis (FRA) system software for data management and analysis. The applied voltage amplitude was 5.0 mV at frequencies ranging between 1 and 100 kHz for EIS measurement. The conductivity was calculated by:

$$\sigma = \frac{1}{R_b} \times \frac{\lambda}{A}$$

where R_b is the bulk resistance from AC impedance, A the area of electrode and l the film thickness.

Scanning Electron Microscopy

The morphology of corrosion surface was observed using a Philips XL30 scanning electron microscopy. Tafel plot and the impedance measurements were recorded until their parameters reached a steady state value, then the specimens were removed, cleaned, dried and examined using SEM.

Results and Discussion

Quasi-Steady State Polarisation Curves

Potentiodynamic anodic and cathodic polarisation curves of alloy steel around E (I = 0) in the active potential region were recorded at 10 mVs^{-1} to allow steady-state current. These curves are given in a semi-logarithmic form (Figure 2) from the extrapolation of the Tafel lines corrosion potentials (E_{corr}) and corrosion current densities (I_{corr}) for the VMNI medium sterile and VMNI containing *Desulfovibrio* sp. The corresponding values are given in Table 2. The data clearly show that the addition of the *Desulfovibrio* sp. influenced the E_{corr} and I_{corr} with the parameters changing drastically after three days of exposure. The high value of E_{corr} decreased the corrosion current density (I_{corr}) and polarisation resistance (R_p). It is also clear that corrosion was energetically favoured in the *Desulfovibrio* sp. inoculated in VMNI medium compared to VMNI sterile. Figure 3 shows the influence of the VMNI containing *Desulfovibrio* sp. for the different exposure periods. As the Tafel plots of the quasi-steady polarisation curves of alloy steel show the open circuit corrosion potentials, E_{corr} drifted to more positive values (E_{corr} is shifted to much less anodic values). These results indicated that the corrosion potential increased with time. The corrosion current densities, I_{corr}, as given in Table 3, showed a slight decrease from 0.368 $\mu A.cm^{-2}$ after three days to 0.293 $\mu A.cm^{-2}$ at 8 days exposure to VMNI containing *Desulfovibrio* sp. This may be due to the instantaneous dissolution of Fe that preceded the sulphide precipitation.

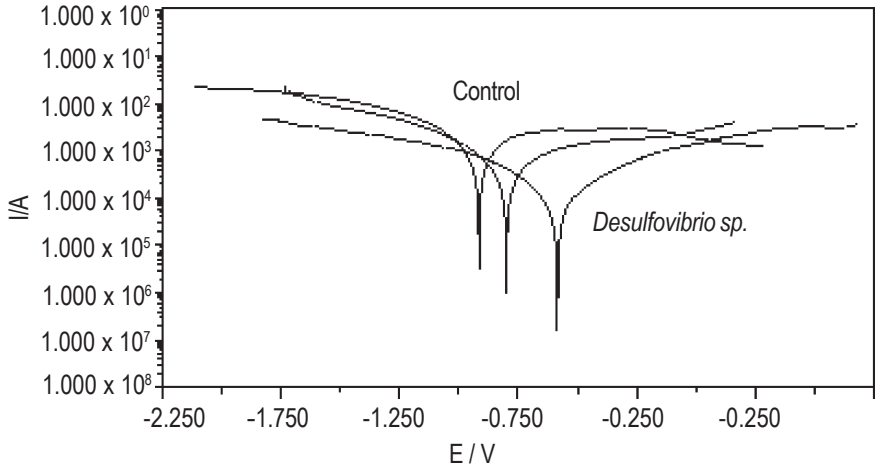


FIGURE 2: Tafel Plots of the Quasi-Steady Polarisation Curves of Alloy Steel in the VMNI Medium Sterile and VMNI Containing *Desulfovibrio* sp. After Periods of Three Days

TABLE 2: Electrochemical Parameters (Data from Tafel Analysis) for the Corrosion of Alloy Steel in VMNI Sterile (Control) and VMNI Containing *Desulfovibrio sp.* After Three Days Immersion

Medium	E_{corr} (V vs sce)	β_a (V/dec.)	β_c (V/dec.)	I_{corr} (μA cm^{-2})
VMNI (control)	-1.088	-9.693	0.604	6.051
Desulfovibrio sp. in VMNI	-0.762	0.506	0.386	4.24

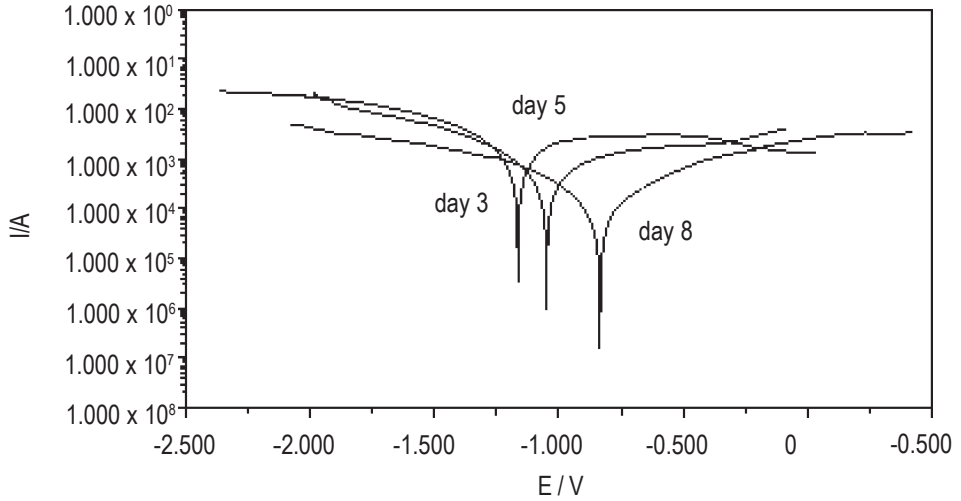


FIGURE 3: Tafel plots of the quasi-steady polarisation curves of alloy steel in the VMNI medium containing of *Desulfovibrio sp*. at scan rate 10 mVs^{-1} and at 35^{o}C: (a) day 3; (b) day 5; (c) day 8

TABLE 3: Electrochemical Parameters (Data from Tafel Analysis) for the Corrosion of Alloy Steel in VMNI Containing *Desulfovibrio sp.* for Different Exposure

Immersion periods	E_{corr} (V vs *sce*)	β_a (V/dec.)	β_c (V/dec.)	I_{corr} (μA cm^{-2})
Day 3	-1.051	0.237	0.31	0.368
Day 5	-1.164	1.0	0.679	0.334
Day 8	-0.835	0.573	0.453	0.293

Corrosion Rates of Electrochemical Cells

Figure 4 shows the corrosion rates of alloy steel in the presence of *Desulfovibrio* sp. and the control for a period of 15 days. The corrosion rates of alloy steel in VMNI containing *Desulfovibrio* sp. rigidly increased after 4 days of exposure compared to the control until a maximum of 5.00E+00 mm/year after 10 days. The corrosion rates then dropped suddenly after 15 days to 2.80E-06 mm/year. The corrosion rates of the control increased slightly after 3 day exposure and reached around ± 1.5E+00 mm/year. These results showed that *Desulfovibrio* sp. could play an important role in biocorrosion and is capable of influencing the process of steel deterioration.

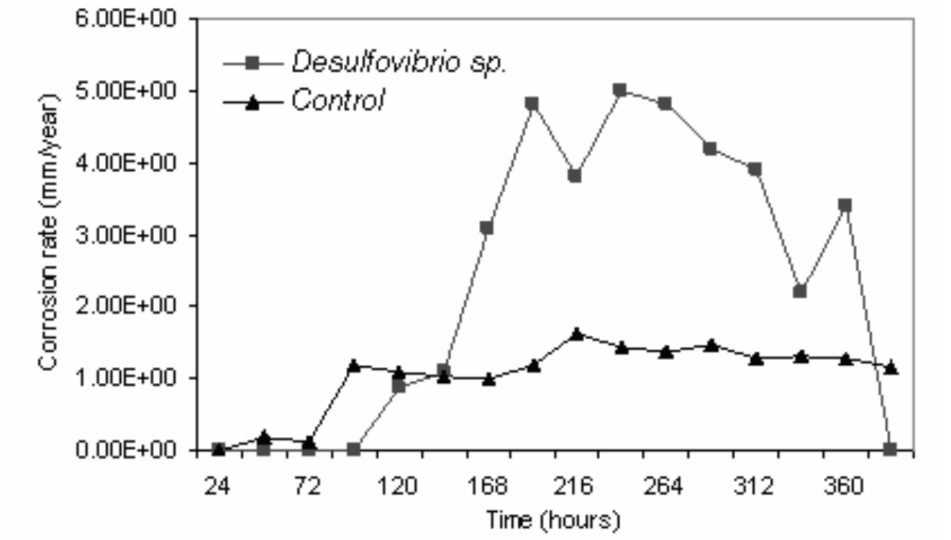


FIGURE 4: Corrosion Rates (Linear Polarisation Resistance) as a Function of Time for Alloy Steel in VMNI (Control) and VMNI Medium Inoculated with *Desulfovibrio sp.*

Impedance Spectroscopy

An impedance spectroscopy study was performed in order to confirm the results obtained with the polarisation test and to complete the analysis. Figure 5 shows EIS plots of Nyquist recorded for the electrode immersed in VMNI sterile (control) and VMNI containing *Desulfovibrio* sp. for 3 days. Respective high frequency zone was expanded and shown in Figure 5. The diameter of Nyquist semicircle of alloy steel immersed in the control was greater than the VMNI containing *Desulfovibrio* sp. suggesting that after a 72-h immersion period, *Desulfovibrio* sp. increased the corrosion rate of alloy steel.

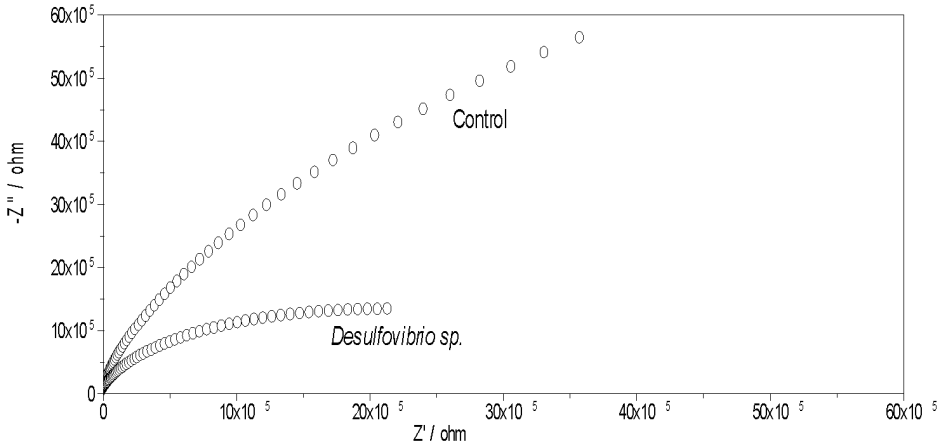


FIGURE 5: EIS Plots of Alloy Steel Coupons Immersed in VMNI Sterile (Control) and VMNI Containing *Desulfovibrio* sp. After Periods of Three Days

Results in Figure 6 show well separated semicircles of Nyquist plots of the electrode immersed in the VMNI medium containing *Desulfovibrio* sp. immersed for different periods. The diameter of Nyquist semicircle plots decreased on increasing the immersion period from 120 h to 192 h. The great decrease in R_p with exposure time indicated an increase in corrosion activity. This could be due to the deterioration of passive layer by *Desulfovibrio* sp. It is clear that the presence of *Desulfovibrio* sp. increased the corrosion rate of alloy steel. However, the diameter of the Nyquist plot increased drastically after 15 day immersion, that is 9.57E+07 ohm and the corrosion rate also decreased sharply to 2.80E-06 mm/year (Table 4). This may have been a result of one or more of the following: (a) the presence of a biofilm acting as a barrier to the diffusion of corrosion products and thereby suppressing the process of metal dissolution; (b) the bacteria producing a metabolic product that acts as corrosion inhibitor for alloy steel. However, earlier works showed that the corrosion rate of alloy steel in the presence of *Desulfovibrio* sp. increased after 72 hours of immersion and decreased after a 15 day immersion. This may be due to the growth phase of SRB. Normally, the *Desulfovibrio* sp. would grow after three days of incubation in VMNI medium and then will be decreased (mortality) after 15 days incubation.

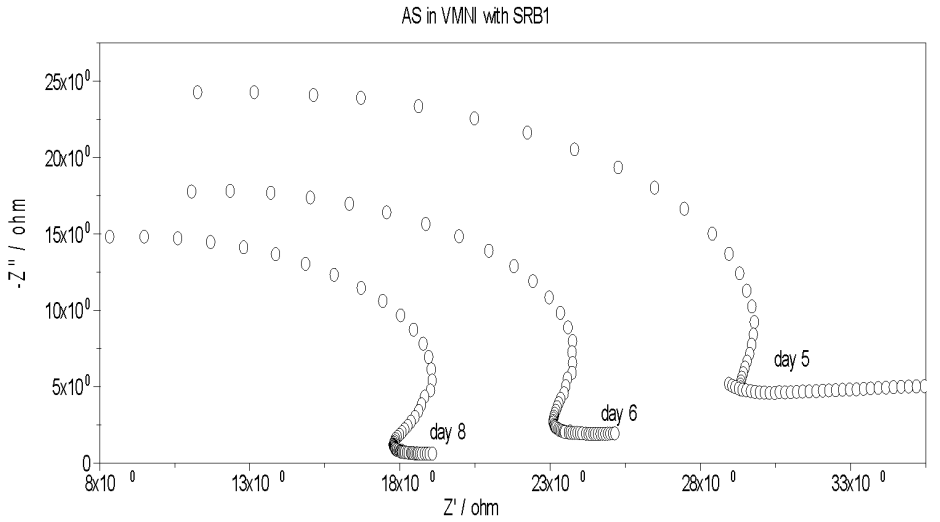


FIGURE 6: Nyquist Plots of EIS Spectra for Alloy Steel Immersed in VMNI Containing *Desulfovibrio* sp. Over a Period of 5, 6 and 8 Days

TABLE 4: Electrochemical Parameters Derived from EIS Plot and Tafel Analysis of Alloy Steel Immersed in VMNI Containing *Desulfovibrio* sp

Time (hour)	Corrosion rate (mm/year) (mm/year)	R_p (ohm) R_p (ohm)	Conductivity (mS/cm) (mS/cm)
120	8.90E-01	1.90E+01	4.34E-02
144	1.10E+00	4.22E+01	2.01E-02
192	4.80E+00	2.40E+01	3.53E-02
360	280E-06	9,57E+07	8,85E-08

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) examination of biofilm on the alloy steel's surface showed the micrograph of *Desulfovibrio* sp. after one day immersion of about 5-6 μm long (Figure 7). Figure 8 shows micrographs of the alloy steel samples after 15 days of exposure in the VMNI sterile and VMNI containing *Desulfovibrio* sp., after the removal of the corrosion products. The micrograph of VMNI medium inoculated with *Desulfovibrio* sp. (Figure 8 (b)) shows clearly a bigger size single pitting over the surface of the steel samples compared to a smaller pitting distributed over the surface of the VMNI sterile (Figure 8(a)). In the case of alloy steel in sterile VMNI, there was minor pitting as is considered as localised corrosion distributed on the surface. The observed damage was subsurface shapes typical of conventional chloride-induced pitting, which may indicate that corrosion was initiated as under-deposit corrosion. The presence of

Desulfovibrio sp. in VMNI medium apparently caused localised corrosion in which larger pits were observed. This agreed well with the E_{corr}, R_p and corrosion rate data.

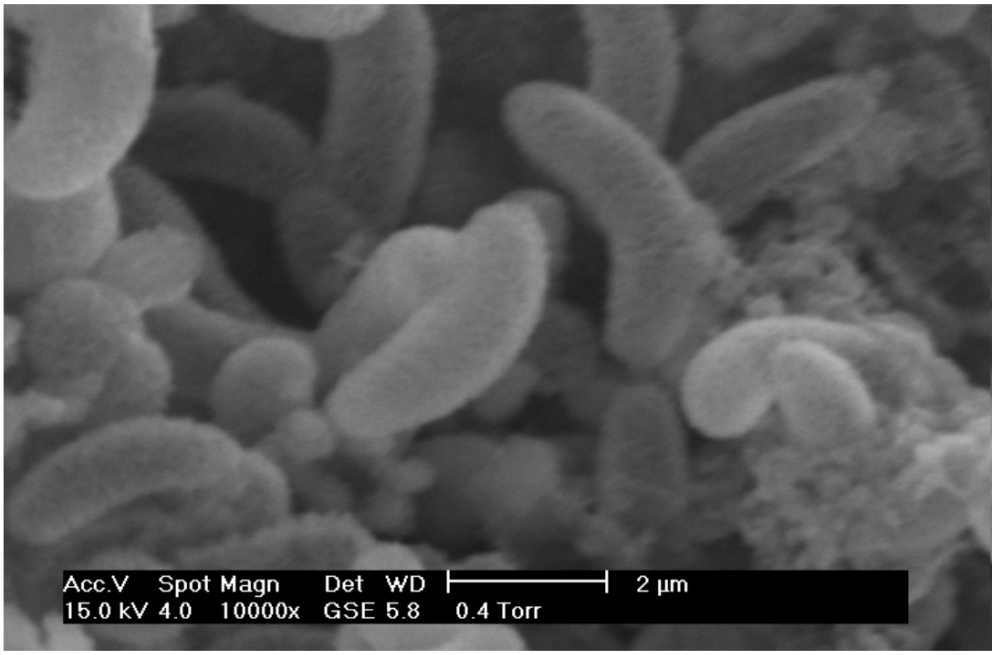
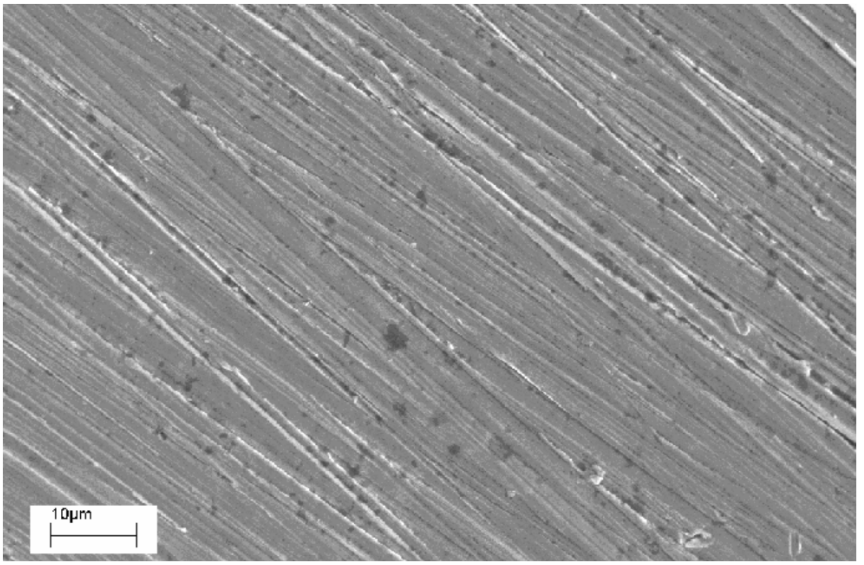
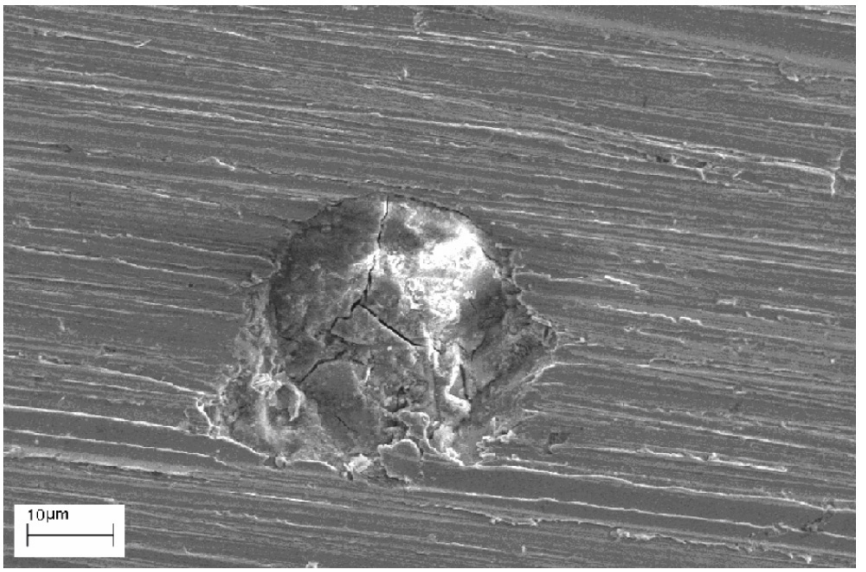


FIGURE 7: SEM Micrographs of Alloy Steel Surface After One Day of Exposure to the VMNI Medium Inoculated with *Desulfovibrio* sp. (Magnification 10,000X)



(a)



(b)

FIGURE 8: SEM Micrographs of Alloy Steel Samples After 15 Days of Exposure to (a) VMNI Sterile and (b) VMNI Containing *Desulfovibrio* sp. (Magnification 2,500X)

The electrochemical impedance spectra obtained from alloy steel in the presence of *Desulfovibrio* sp. showed great reduction in R_p compared to those obtained in the sterile (control) (Figure 5). The alteration of the oxide layer structure facilitated the formation of localized corrosion when another aggressive anion, such as chloride was present. Furthermore, the reduced sulphur compounds in the absence of chloride could induce pitting corrosion of passive film. Also it is well-known that deposits can enhance localized corrosion of alloy steel. The corrosion reaction could be induced at an anodic site under deposit and would be supported by a cathodic reaction of metal sulphides deposits and significant localized corrosion would be initiated. The formation of an imperfect oxide layer on passive metal layer in the presence of sulphur has been reported (Videla et al., 1999). Thus, it is reasonable to assume that the growth of *Desulfovibrio* sp. not only reduced the passive film thickness by forming metal sulphides but also initiated localized corrosion.

Conclusions

In conclusion, Tafel plots and EIS technique can be used to study the mechanisms of corrosion and surface passivity. The corrosion rates of alloy steel in VMNI containing *Desulfovibrio* sp. were obviously increased compared to the control. The Nyquist semicircle of alloy steel immersed in the control was greater than the VMNI containing *Desulfovibrio* sp. The presence of SRB has led to a significant decrease in the value of

R_p and gives rise to localised corrosion. The significant corrosion micropits were observed for alloy steel in the presence of the *Desulfovibrio* sp.

Acknowledgements

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