Electrochemical Impedance Spectroscopy Studies of Stainless Steel Corrosion by Two Strains Marine Sulfate Reducing Bacteria
(Kajian Spektroskopi Elektrokimia Impedans Terhadap Keluli Kalis Karat Oleh Dua Strain Bakteria Penurun Sulfat Marin)

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ABSTRACT

This research was conducted to compare corrosion potential of two strains of marine sulfate reducing bacteria (SRB1 and SRB2) on their effect of biocorrosion on stainless steel AISI304 using electrochemical techniques. This study was carried out using Electrochemical Impedance Spectroscopy (EIS) to determine the impedance value (passivity, Rp) and corrosion rate. An electrochemical measurement showed that the value of Impedance for Stainless steel in SRB culture is much smaller compare to VMNI medium (control), indicating a degradation of metal was occurred. SRB2 showed more aggressiveness on corrosion compare to SRB1 as the impedance value, Rp of stainless steel in SRB2 culture is lower compare to SRB1. In conclusion, EIS techniques given a good results to study mechanisms of corrosion and surface passivity.

Keywords: Sulfate-reducing bacteria, stainless steel, electrochemical impedance spectroscopy

ABSTRAK

Kajian ini telah dijalankan bagi membandingkan keupayaan kakisan dua strain bakteria bakteria penurun sulfat marin (SRB1 and SRB2) terhadap kesan biokakisan pada AISI304 keluli kalis karat menggunakan teknik elektrokimia. Kajian ini dijalankan menggunakan Spektroskopi Elektrokimia Impedans (EIS) bagi mengenalpasti nilai kerintangan (Rp) dan kadar kakisan. Pengukuran elektrokimia menunjukkan nilai kerintangan bagi keluli kalis karat dalam kultur SRB lebih rendah berbanding medium VMNI (kawalan), menunjukkan kemerosotan logam telah berlaku. SRB2 menunjukkan lebih aggresif mengkakis berbanding SRB1 yang mana nilai kerintangan, Rp dalam kultur SRB2 lebih rendah berbanding kultur SRB1. Kesimpulannya, teknik EIS menunjukkan hasil yang baik bagi mengkaji mekanisma kakisan dan kerintangan permukaan.

Katakunci: Bakteria penurun sulfat, keluli kalis karat, spektroskopi elektrokimia impedans

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INTRODUCTION

The biofouling and biodeterioration of man-made materials, including metal and their alloys, due to the biofilm formation has great environmental and economical implications. Many industrial sectors such as gas, oil, nuclear power, shipping, aircraft, chemical and civil engineering super potential pollution problems, health and safety hazards and substantial financial losses as a result of biofilm development (Beech 2004). Bacteria–metal interactions in aqueous solutions are important in biofilm formation, biofouling and biocorrosion problems in the natural environment and engineered systems (Sheng et al. 2008). Much research has focused on microbially-influenced corrosion (MIC) of metals and alloys in the marine environment. Some bacteria have been reported to accelerate the corrosion of metals owing to the metabolism or the presence of bacteria by changes in the microchemistry of the surface due to the bacterial metabolic activity and biofilm formation (Crist´obal et al. 2006, Liu et al. 2007, Sheng et al. 2007). Problems associated with biocorrosion due to the formation of biofilms are widespread and have serious economic and safety implications (Beech 1996).

Bacteria and cyanobacteria are characteristic producers of biofilms where many individuals live within an Extrapolymeric Substances (EPS) matrix. Initial attachment of cells is reversible but becomes permanent after the secretion of adhesive polymers. Bacteria live within the deeper parts of this matrix, generating organic compounds by sulphate reduction (Wotton 2004). Various specific mechanisms, including differential aeration, selective leaching, underdeposit corrosion, cathodic depolarization and metal ion binding by bacterial EPS, have been proposed to interpret the MIC (Yuan et al. 2007).

Sulfate-reducing bacteria (SRB) are a group of phylogenetically diverse anaerobes that perform the dissimilatory reduction of sulfur compounds including sulfate, sulfite, thiosulfate and even sulfur to form sulfide (Yu Chan et al. 2002, Lloyd et al. 2001, Chang et al. 2008, Muyzer & Stams 2008). These bacteria are generally associated with the area underneath the main corrosion shell since they require anaerobic conditions where it can occurs on surface due to the presence of other aerobic bacteria producing anoxic pockets (Seth & Edyvean 2006). SRB are the major biological source of biogenic sulfide, which causes microbiologically influenced corrosion (MIC) in anoxic habitats (Dzieriewicz et al. 1997, Chang et al. 2008).

The sulfate-reducing bacteria exhibited higher corrosion rate, this is attributed to SRB have been documented to show aggressive corrosion with many metals under anaerobic conditions, they are ubiquitous and easy to culture, sulfate-containing environments they produce hydrogen sulfide that can react with stainless steel (Xu et al. 2007). In the presence of SRB the main corrosion products formed on the iron surface are ferrous sulfides, which can be protective of or aggressive to the underlying metal (Beech 2003). SRB can promote corrosion by reducing sulfate to sulfide, which may in turn oxidize hydrogen to give hydrogen sulfide, and this may react further to produce ferrous sulfides or sulfuric acid, which also contributes acid corrosion (Beech 2003, Seth & Edyvean 2006,). This corrosion reaction lead to formation of hydrogen sulfide, creating the ''rotten egg'' smells, and the possibility of production of black slime. (Seth & Edyvean 2006, Muyzer & Stams 2008, Sahrani et al. 2008).

Many of the biofilms that adsorb on metallic surfaces immersed in natural aqueous environments are nonconducting and EIS techniques are potentially useful in their presence (Feron 1991, Mansfeld et al. 1990, Dexter et al. 1991, Gonzalez et al. 1998). Electrochemical

impedance spectroscopy retains all the advantages of traditional direct-current (dc) methods. It is sensitive, can be conducted in situ, and often does not require artificial accelerating factors for testing, such as increased temperature and concentration (Dexter et al. 1991). One more of the advantages of EIS are the use of very small signals, which do not disturb the electrode properties and associated microbiology. EIS also offers the opportunity to characterize the corrosion reaction and measure the corrosion rate in electrolytes with low conductivity. This study was conducted to compare corrosion potential of two strains of marine sulfate reducing bacteria (SRB1 and SRB2) on their effect of biocorrosion on stainless steel AISI304 using electrochemical techniques.

MATERIALS AND METHODS

Bacteria were isolated from biofilms and corrosion product forms on steel coupons immersed in seawater vacinity at Pasir Panjang, Port Dickson Negeri Sembilan. Samples were collected aseptically using 1000mL Scott bottles and plastic containers in semi-anaerobic condition. Pour and streak method plate technique was carried out to attempt isolate pure colony or consortium comprising two or three known species. VMNI medium was use as enrichment medium for anaerobic bacteria especially for sulfate reducing bacteria group (Table 1). The bacteria cultures were maintained in the lab in the VMNI medium. The pH was adjusted to 7.2 using 1.0 M NaOH and then was autoclave at $121°C$. SRB Bart kits were used to determine the culture containing of SRB group.

Stainless steel (AISI 304) was used as sample coupons (Figure 1). This coupon was prepared according to ASTM corrosion testing standards to cylindrical (9.5mm diameter x 6.0 mm height). For electrochemical test, cylindrical coupons mounted in polyester resin were used. All coupons are polished with a series of grit SiC papers (grade 400, 600, 800 1000, 1200), followed by ethanol degreasing. After further polishing using alumina powder (0.1µm and 0.6 µm), the coupons were cleaned, dried and stored in desiccators until used. A copper wire was soldered at the rear of the electrode which was housed in a glass tube to protect it from the test medium.

Electrochemical Impedance experiment was carried according to ASTM standard cell with a three electrode system: stainless steel as working electrode, a platinum rod was used as counter electrode and saturated calomel electrode as reference electrode. The working electrode was immersed in 300 ml VMNI medium that act as electrolyte solution. The electrochemical cell was connected to Solatron 1286 Electrochemical Interface that were connected to Solatron 1255 Frequency Respons Analyzer and a PC was used for data recording. The applied voltage amplitude was 10 mV at frequencies range between 1 and 100 kHz for EIS measurement. Reading will be take for 15 days and were always obtained at least in triplicate.

During 15 days of immersion, the number of bacteria growth were observed and recorded using dilution and plate counting technique and the number of growth were calculated using the below equation :

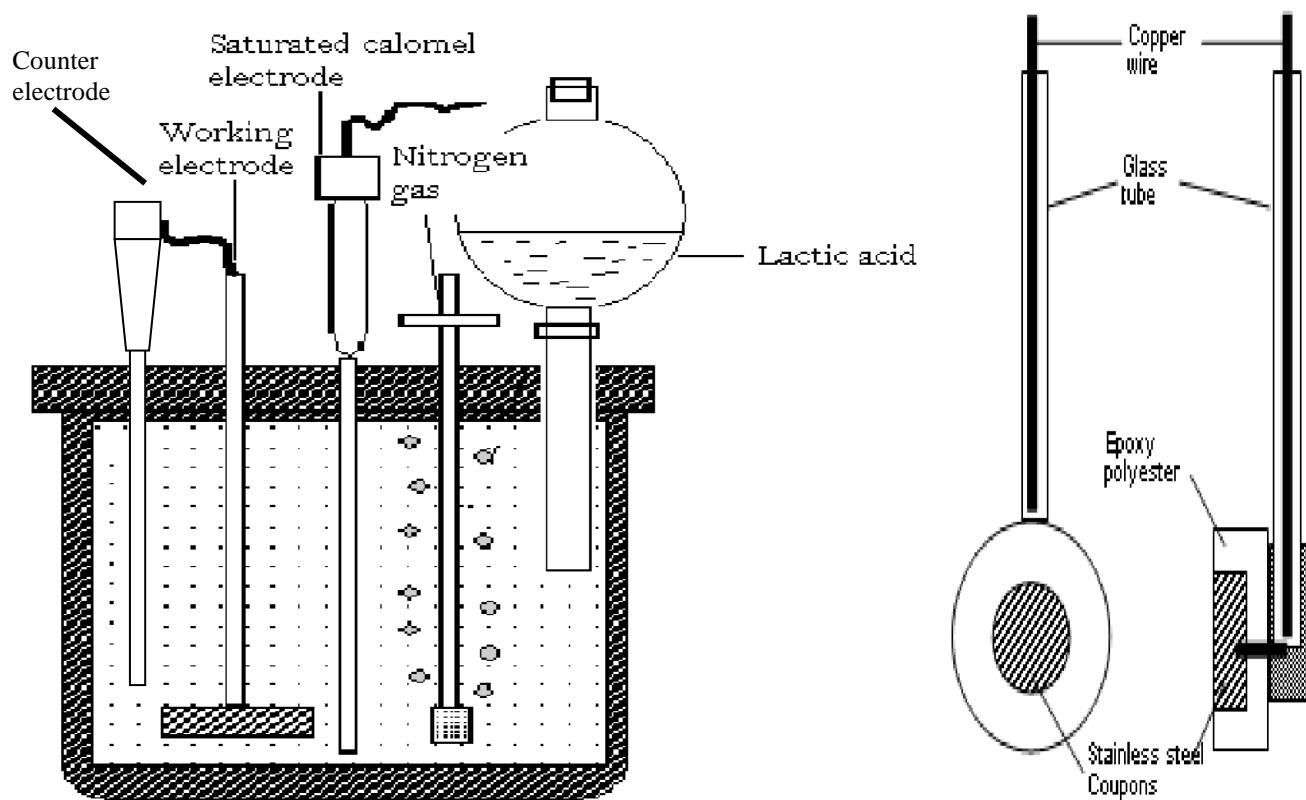$$CFU = \frac{No.\,of\,colony\,\times\,dilution\,factor\,\times\,1ml}{0.1\,ml}$$

Figure 1. (a) Schematic diagrams of electrochemical cell and (b) Stainless steel concentric electrode (as working electrode)

Table 1: Composition of VMNI medium

Chemical Substances	Composition (g/L)
KH_2PO_4	0.5
NH_4Cl	1.0
Na_2SO_4	4.5
$Na_3C_6H_5O_7 \cdot 2H_2O$	0.3
$CaCl_2.6H_2O$	0.04
$MgSO_4.7H_2O$	0.06
Casamino Acid	2.0
Triptone	2.0
sSodium lactate	5.0
Ascorbic Acid	0.1
Thyglicolic Acid	0.1
$FeSO_4.7H_2O$	0.5
Unsur Surih (stok larutan)	1.0ml
Vitamin (stok larutan)	2.0ml

RESULTS AND DISCUSSION

**Electrochemical Impedance Spectroscopy**

The impedance spectra of stainless steel AISI 304 in the sterile VMNI medium and VMNI medium inoculated with SRB1 and SRB2 as a function of time are shown in Figure 3. The diameters of the semicircles in the Nyquist plot for the stainless steel in bacteria inoculation is smaller compare to sterile VMNI medium throughout 15 day of the immersion. The differences of Rp value between these two conditions can be seen in Table 2. From 1st to 15th day of immersion, SSVCONTROL show a significant change where the value drops from 3807.2 ohm to 2441.4 ohm from day 1st day to the 5th day. Then the values become constant at the range of 2375.8 ohm to 2068.0 ohm.

For SSSRB1 stainless steel, it shows a very large decline in the Rp value from day 1 to day 7 of immersion where it goes from 3678.2 ohm to 1684.8 ohm (Figure 3). From day 9 to day 11, declining of Rp still happen but in a slower rate, where it drops from 1343 ohm to 1262 ohm. But on the 13th and 15th day, Rp become constant between the value of 1130.3 ohm and 1116.7 ohm.

SSSRB2 show the same pattern of decline in the Rp value with SSSRB1, where it goes from 3736.4 ohm on day 1 to 1410.3 ohm on day 7. The declining of Rp value still happen from day 9 until day 15 as it goes from 1171.1 ohm to 988.8 ohm. Compare to SRB1, SRB2 showed much more declined in Rp from day 1 to day 15.

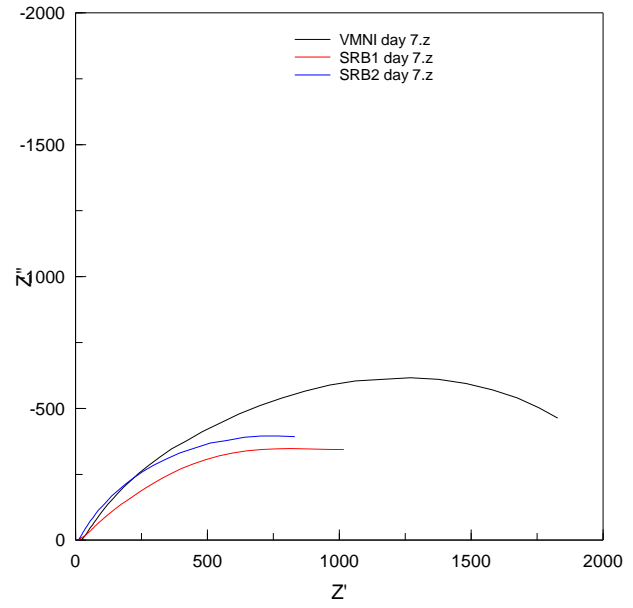Table 2: Value of EIS of Stainless Steel in VMNI medium, SRB1 and SRB2

Day	1	3	5	7	9	11	13	15
VMNI	3807.2	3220.7	2441.4	2375.8	2243.2	2196.0	2131.0	2068.0
SRB1	3678.2	2051.9	1783.2	1684.8	1343.0	1262.0	1130.3	1116.7
SRB 2	3736.4	1723.9	1694.9	1410.3	1171.1	1116.2	1072.2	988.8

Day 1

Day 3
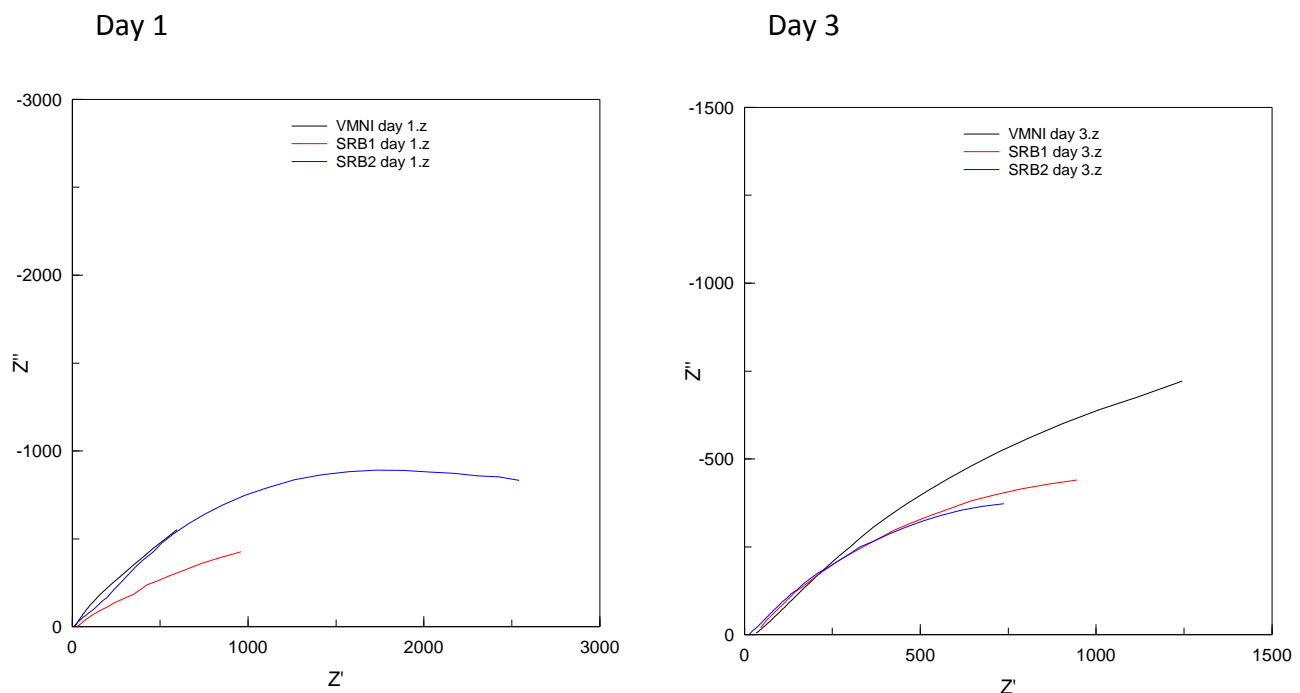
Day 5

Day 7

Day 9
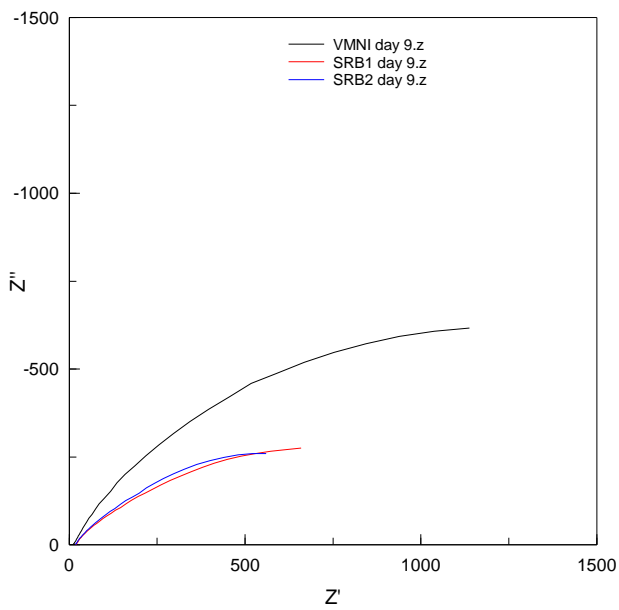

Day 11

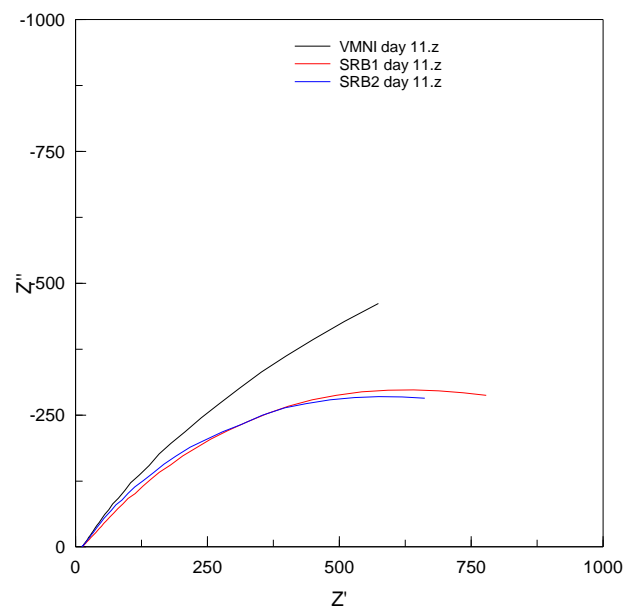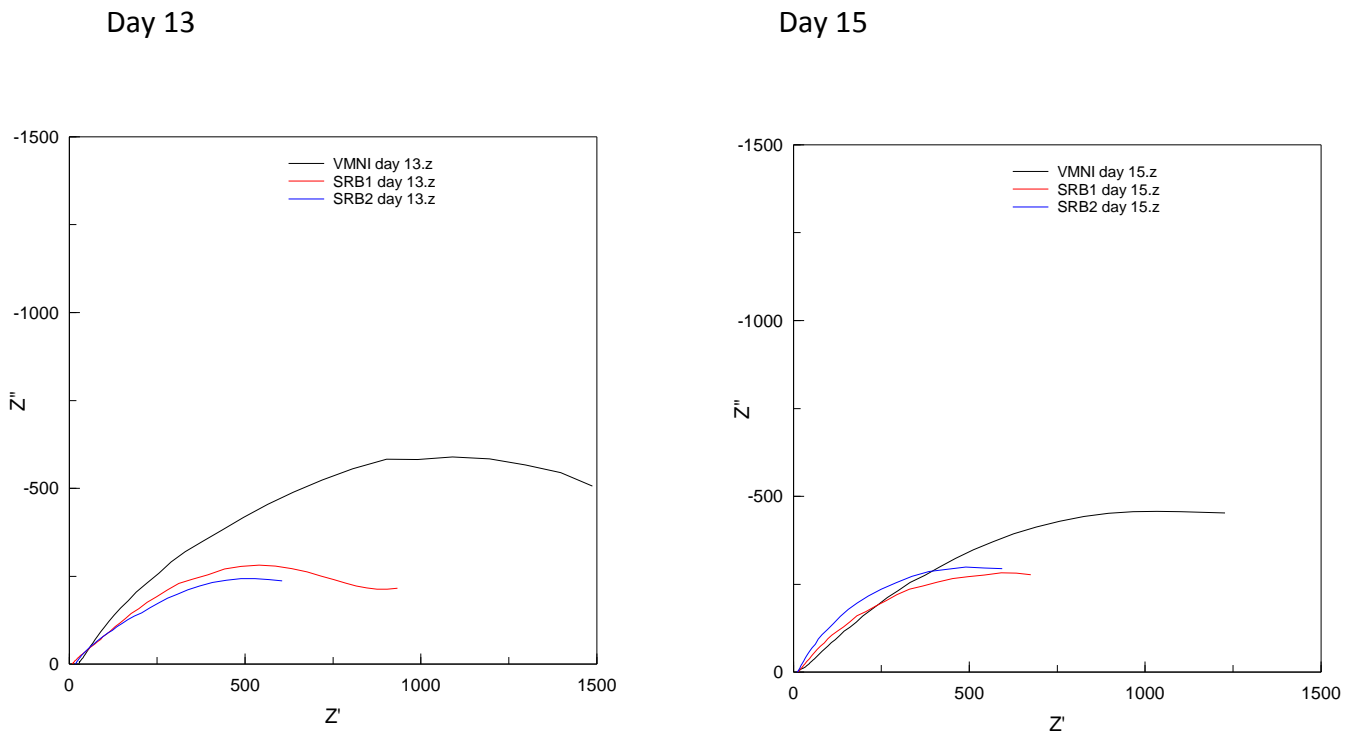Day 13   Day 15
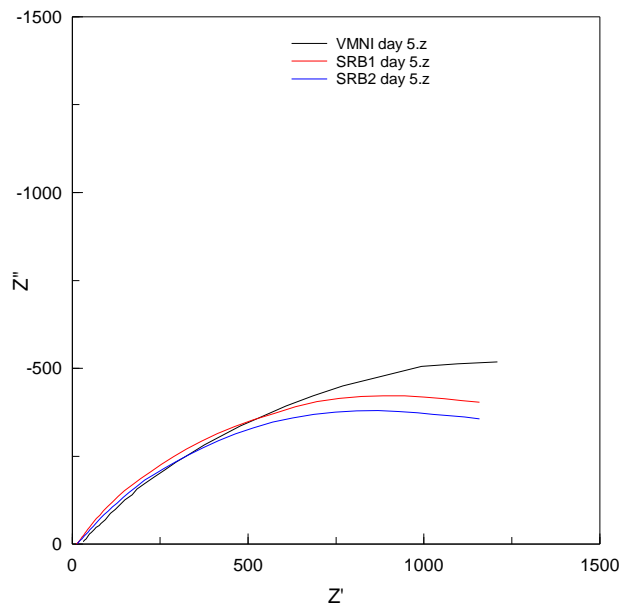
Figure 3: Nyquist plost for corrosion of stainless steel in sterile VMNI (control), VMNI inoculated with SRB1 and SRB2.

Rp value for SSSVCONTROL showed a drop that maybe contributed by the general corrosion in the solution. Stainless steel AISI 304 had been known to have high resistance toward chemical based corrosion by the formation of thin hydrogen film that protects the steel from corrosion (Feron 1991). The alteration of the hydrogen layer structure facilitates the formation of localized corrosion by the present of an aggressive anion such as chloride (Ismail et al. 1999). The complexity of the VMNI medium creates a very complex reaction between the electrolyte and the surface of stainless steel (Sahrani et al. 2009), also contribute to the breakdown of the passive layer. Steel that was exposed induces corrosion process to happen leading towards the drop of Rp value. But for a while, the passive layer will be build again and corrosion will be slowed down thus increasing the value of Rp.

The electrochemical impedance spectra obtained from stainless steel in the presence of SRB suggest a greater reduction in Rp in comparison with those obtained in the sterile control experiments. The decrease in impedance lends support to the corrosive effect of the SRB on stainless steel (Sheng et al. 2008). The act of bacteria in reducing the Rp value of steel have been shown in several research using the EIS method (Miranda et al. 2006, Xu et al. 2007, Liu et al. 2007, Chang et al. 2007, Sahrani et al. 2009).

SRB decrease Rp and make it easier for electrode degradation (Liu et al. 2007). The lower polarization resistance in the presence of SRB indicates the lost of protected surface layer (passive layer) thus exposing the specimen surface to corrosive species in solution produced by SRB activities. Reduced sulfur compounds in the complete absence of chloride can induce pitting corrosion of stainless steel (Ismail et al. 1999). During the growth of SRB-biofilm, SO_4^{2-} ions is reduced to HS^- ions which then react with Fe^{2+} ions to form sulphide precipitate in the biofilm that consist of FeS, FeS_2 and Fe_9S_8 (Sahrani et al. 2009, Dong et al. 2011) where this substances is highly electron conductive. This deposits material create a non-homogeneous films of sulfide products that serve as strong cathodes to accelerate the

oxidation of Fe, that would be supported by a cathodic reaction at the metal sulfides deposits and thus initiates localized corrosion (Ismail et al. 1999, Sheng et al. 2008).

The existence of the biological film clogged the contact between solution medium and electrode, creating variance in oxygen content and pH value thus bringing about an environmental change on the electrode surface, so the ostensible corrosion of the basic material was aggravated (Chang et al. 2007). The pH of micro-environment under the biofilm become lower due to the presence of hydrogen sulfide (Sheng et al. 2008) and the formation of mild H_2SO_4 by the usage of hydrogen on stainless steel surface. Cytochrome and hydrogenase on bacterial outer membranes, participate in the reductive dissolution passive films within the interaction between SRB biofilm and passive film and zero valent stainless steel substrate (Xu et al. 2007).

The difference value of Rp that were recorded between the two bacteria mostly depends on the corrosiveness of the metabolomics substances that were produced by the bacteria and rate of production of biofilm that directly proportional with the bacteria growth. The capacitance of the interface between the electrode face and the medium increased rapidly on account of the bigger specific surface, because of a certain thickness of the film (Chang et al. 2007). From Figure 4, it shows that SRB2 produce more corrosive substances and higher rate of biofilm formation as the Rp of stainless steel in SRB2 is lower than SRB1. The growth curve also showed that SRB2 can adapt and grow much faster than SRB1. Thus can be expected that the more thickness of biofilms cover the surface of stainless steel in SRB2 immersion compared to SRB1.

In day 1 the corrosion process is still low correspondence as the lower number of SRB, thus the formation of biofilm, intake of cathodic hydrogen and precipitation of ferum sulphate are also lower compare to the formation of passive layer on stainless steel, it's showed by a high value for Rp at 3678.2 for SRB1 and 3736.4 for SRB2. The sudden drop was occurred on the 3rd day that was observed on all of stainless steel in the media, maybe cause by the corrosiveness of the VMNI medium itself. But the Rp value for stainless steel in SRB1 and SRB2 culture is lower (2051.9 ohm and 1723.9 ohm respectively) compare to the control (3220.7 ohm).

The decreasing value of Rp was still occurred from day 3rd to day 11th for both SRBs. Number of SRB increase from day 3 to day 9. As it grow in number, also the utilization of hydrogen increase, thus removal of hydrogen on steel surface increase. The passive film was removed from steel and exposing the steel to corrosion cause the dropping of the Rp value from 1783.2 ohm to 1343.0 ohm for SRB1 and 1694.9 ohm to 1171.1 for SRB2. The effect of FeS depolarization also added to the higher rate of corrosion as the reduction of H_2S by SRB take place. The greater of SRB activities, the more of S^{2-} created and also more FeS will be form.

The increasing number of SRB1 and SRB2 from day 9th and 11th that were recorded does not gave a big impact on the Rp reading for both stainless steel. The nutrient limitation is one of the factors for this condition, as the nutrient only support metabolomics activity for growth only. On day 13th onward the declining of number of bacteria do give an impact on the reading of Rp as the Rp in SRB1 became more linear and no bigger changes happens as mostly only general corrosion taking its effect. The building up of the passive layer is directly proportional with the dissolution of metal.
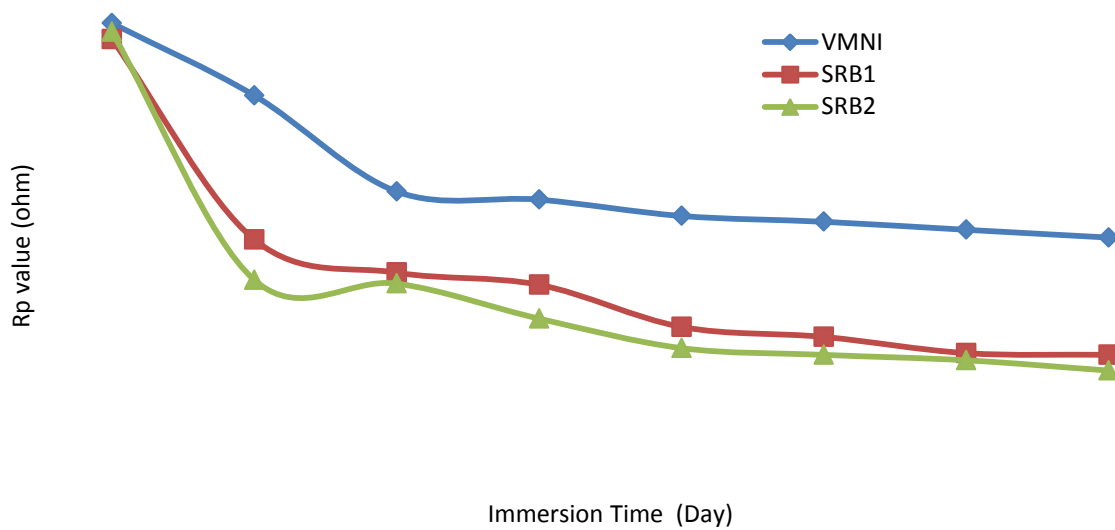
Figure 4: EIS graph for stainless steel in VMNI medium, SRB1 and SRB2 medium for 15 days immersion

Bacteria Growth Curve

SRB2 showed a faster growing rate compare to SRB1 (Figure 5). In day 1 to day 7 the increments is a little bit slow as it goes from 500 CFU/ml to 900 CFU/ml for SRB1 and 450 CFU/ml to 1000 CFU/ml for SRB2. A sudden increase happens on day 7th and 11th where the highest number was recorded, 33000 CFU/ml for SRB1 and 36000 CFU/ml for SRB2. The numbers keep on declining after passing the 11th day until the 15th. SRB1 showed a decline to 29000 CFU/ml on day 13 and become almost constant until day 15 at 28500 CFU/ml. SRB2 still showed a decline in number as it goes from 30000 CFU/ml to 27000 CFU/ml for day 13 and 15.
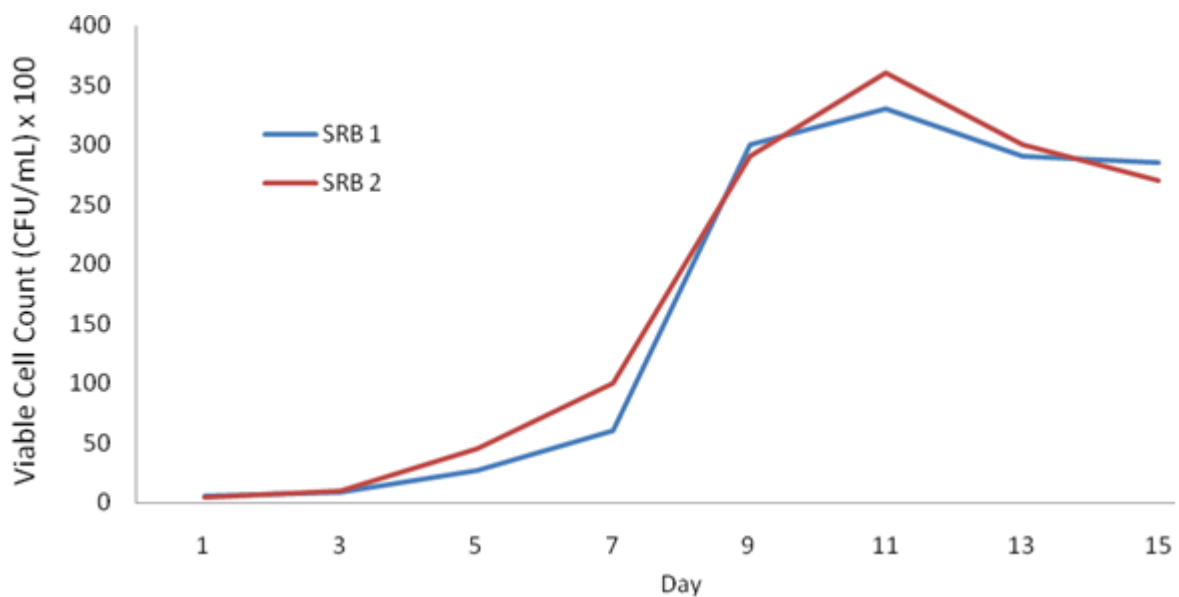

Figure 5. EIS graph for viable cell count SRB1 and SRB2 medium for 15 days immersion

The growth of bacteria for 15 days showed connection between the growth and the decline of Rp value and OCP value. There are several SRB growth phases throughout the 15 days of experiment that is the latent period, the rapid growth period, the stable growth period and the declining period as proposed by Liu et al. 2007. The latent period is where the growth of SRB started and the process of adaptation happen (Figure 5). The formation of biofilm is slower but the intake of nutrients is rapidly happening for growth but in a small number. This is shown from day 1 to day 3.

The rapid growth period can be seen on the 3rd until 7th day of immersion, where the number of bacteria increases 4 times higher. This is where the depletion of nutrients in the medium happen as rapid intake of nutrient was done by a large number of bacteria. Production of metabolomic substances and rate of biofilm formation is higher in this phase. On the 9th day stated the start of the stable growth period where the nutrient intake and growth is at the same rate. The intake of hydrogen for sulphate reduction will be proportional with the production of hydrogen itself. The declining period started when the nutrient is not sufficient to support the growth of bacteria. Even though lactate was supply into the medium, other substances is still low in number. Day 13th to 15th marked the declining in the number of bacteria.

SRB2 show a very good adaptation in the medium and faster growth rate compare to SRB1 as from day 1 to day 7, the number of SRB2 always exceed SRB1. Even though on day 9th SRB1 numbers exceed the number of SRB2, on day 10th we can see that higher number of SRB2 were recorded. The declined in number on day 9 maybe caused by the declined of lactate in the medium as other carbon source have been used for growth, thus after lactate were added, there is a huge increment on day 10 as there are new source of carbon.

CONCLUSION

SRBs play a major role in the corrosion of stainless steel AISI 304. The declining of Rp value in EIS test showed a positive sign of steel passive layer attacked by SRB. The growth rate of SRB2 is higher than SRB1, SRB2 showed more corrosiveness characteristic compare to SRB1 as it's showed by a lower value of Rp. The value of Rp in an experiment corresponded with the growth pattern of SRB but the number of bacteria does not directly affect the corrosiveness of the steel itself. The differences value of Rp that were recorded between two strains bacteria mostly depends on the corrosiveness of the metabolomics substances produced and production rate of biofilm directly influenced by proportional with the bacteria growth.

ACKNOWLEDGEMENTS

REFERENCES

Beech, I., 2004. Corrosion of technical materials in the presence of biofilms — current understanding and state-of-the art methods of study. *International Biodeterioration & Biodegradation* **53**, 177 – 183.

Beech I., 2003. Sulfate-reducing bacteria in biofilms on metallic materials and corrosion. *Microbiology Today.* **30(8)**, 115 – 117.

Beech, I., 1996. The Potential use of atomic force microscopy for studying corrosion of metals in the presence of bacterial biofilms - an overview. *International Biodeterioration & Biodegradation*, 141 – 149.

Chang X.T., Yin Y.S., Niul, G.H., Liu, T., Cheng, S., Sun, S.B., 2007. The electrochemical behaviour of oceanic microbiological influenced corrosion on carbon steel. *Acta Metall. Sin.* **20(5)**, 334-340.

Chang Y., J., Chang Y., T. & Hung C., H. 2008. The use of magnesium peroxide for the inhibition of sulfate-reducing bacteria under anoxic conditions. *Jour. Ind. Microbiol. Biotechnol*. **35**, 1481–1491.

Crist´obal, A. B., Arenas, M.A., Conde, A., De Damborenea, J., 2006. Corrosion of stainless steels covered by exopolymers. *Electrochimica Acta*. **52**, 546–551.

Dexter, S.C., Duquette, D.J., Sierbert, O.W., Videla, A., 1991. Use and limitations of electrochemical techniques for investigating microbiological corrosion, *Corrosion* **47**, 308-318.

Dong, Z.H., Shi, W., Ruan, H.M., Zhang G.A., 2011. Heterogeneous corrosion of mild steel under SRB-biofilm characterised by electrochemical mapping technique. *Corrosion Science*.

Dzieriewicz, Z., Cwalina, B., Chodurek, E., Wilczok T., 1997. The relationship between microbial metabolic activity and biocorrosion of carbon steel. *Res. Microbial*. **148**, 785-793.

Sahrani, F.K., Ibrahim, Z., Aziz, M., Yahya, A., 2008. Open circuit potential study of stainless steel in environment containing marine sulphate-reducing bacteria. *Sains Malaysiana* **37(4)**, 359–364

Sahrani, F.K., Ibrahim, Z., Aziz, M., Yahya, A., 2009. Electrochemical impedance spectroscopy and surface studies of steel corrosion by sulphate-reducing bacteria. *Sains Malaysiana* **38(3)**, 359-364

Feron, D., 1991. Chemical and electrochemical aspects of the corrosion of stainless steels in the presence of sulphate reducing bacteria. *Microbially Influenced Corrosion and Biodeterioration* **51**, 73-83.

González, J.E.G., Santana, F.J.H., Mirza-Rosca, J.C., 1998. Effect of bacterial biofilm on 316 SS corrosion in natural seawater by EIS. *Corrosion Science* **40(12)**, 2141-2154.

Ismail Kh.M., Jayaraman, A., Wood, T., K., Earthman, J., C., 1999. The influence of bacteria on the passive film stability of 304 stainless steel. *Electrochimica Acta*. **44**, 4685 – 4692.

Liu, J., Liang, X., Li, S., 2007. Effect of sulphate-reducing bacteria on the electrochemical impedance spectroscopy characteristics of lCrlSNi9Ti. *Journal of University of Science and Technology Beijing*. **14(5)**, 425.

Lloyd, J.R., Mabbett, A.N., Williams, D.R., Macaskie, L.E., 2001. Metal reduction by sulphate-reducing bacteria: physiological diversity and metal specificity. *Hydrometallurgy* **59**, 327–337.

Mansfeld, F., Tsai, R., Shih, H., Little, B., Ray, R., Wagner, P., 1990. Paper No.109, Presented at .*Corrosion/9~* NACE:Houston, TX

Miranda, E., Bethencourt, M., Botana, F.J., Cano, M. J., Sa´nchez-Amaya, J.M., Corzo, A., Garcı´a de Lomas J., Fardeau, M.L., Ollivier, B., 2006. Biocorrosion of carbon steel alloys by an hydrogenotrophic sulfate-reducing bacterium Desulfovibrio capillatus isolated from a Mexican oil field separator. *Corrosion Scienc*e **48**, 2417–2431

Muyzer, G., Stams A.J.M., 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Microbiology* **6**, 414-454.

Seth, A., D., Edyvean R.G.J., 2006. The function of sulfate-reducing bacteria in corrosion of potable water mains. *International Journal of Biodeterioration and Biodegradation* **58**, 108-111.

Sheng, X., Ting, Y., P., Pehkonen, S.O., 2007. The influence of sulphate-reducing bacteria biofilm on the corrosion of stainless steel AISI 316. *Corrosion Science* **49**, 2159–2176.

Sheng, X., Ting Y., Pehkonen S.O., 2008. The influence of ionic strength, nutrients and pH on bacterial adhesion to metals. *Journal of Colloid and Interface Science* **321**, 256–264.

Wotton, R.S., 2004. The Essential Role of Exopolymers (EPS) In Aquatic Systems Oceanography and Marine Biology **42**, 57–94.

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. *Materials Science and Engineering* **443**, 235 – 241.

Yuan, S., J., Choong, A.M.F., Pehkonen S.O., 2007. The influence of the marine aerobic Pseudomonas strain on the corrosion of 70/30 Cu–Ni alloy. *Corrosion Science* **49**, 4352 – 4385.

Yu Chan, K., Chong, X., Fang, H.P., 2002. Anaerobic electrochemical corrosion of mild steel in the presence of extracellular polymeric substances produced by a culture enriched in sulfate-reducing bacteria. *Environ. Sci. Technol* **36**, 1720-1727.