



## American-Eurasian Journal of Sustainable Agriculture

ISSN: 1995-0748

JOURNAL home page: <http://www.aensiweb.com/AEJSA>

2015 Special; 9(2): pages 29-34.

Published Online 11 February 2015.

Research Article

### Microbial Corrosion of Carbon Steel by Tropical Environment Consortium Bacteria Containing SRB

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Received: 31 December 2014; Revised: 26 January 2015; Accepted: 28 January 2015

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#### ABSTRACT

There are various cases involving microbiology influenced corrosion (MIC) that responsible for corrosion problems, especially in oil and gas industry. Sulphate reducing bacteria (SRB) that implicated in MIC mechanisms, was contributing as a main problem for localized corrosion. The aim of this paper is to study the effects of consortium bacteria containing SRB (C-SRB) from tropical environment on microbial corrosion of carbon steel. The analyses were carried out by using weight loss and potentiodynamic polarization methods. Surface morphology on carbon steel surface and biofilm formation from this C-SRB activities were characterized by variable pressure scanning electron microscopy (VPSEM) and energy dispersive X-ray spectroscopy (EDX). Results from both weight loss and potentiodynamic polarization methods confirmed that the corrosion rate of carbon steel, which is inoculated with C-SRB is higher than free C-SRB in same VMNI medium used. C-SRB had seen to effect the carbon steel surface due to its metabolism activities at a particular period of time and this activities was confirmed by morphology analysis (VPSEM) and EDX. Based on this study it is concluded that C-SRB actively involved as microbial corrosion on carbon steel surface due to its nature metabolism activities.

**Keywords:** Carbon steel; Potentiodynamic polarization; Microbiologically influenced corrosion; C-SRB

#### INTRODUCTION

Sulphate reducing bacteria (SRB) is one of the most damaging microorganisms in oil and gas industry. SRB is an anaerobic bacteria and grow in the absence of oxygen at temperature range 25-60°C. This bacteria used sulfate ion as terminal electron acceptor and produced hydrogen sulfide (H<sub>2</sub>S) as main product [5,6,7]. SRBs are the main reason to cause the MIC by accelerating the corrosion rate, biofilm formation and pitting corrosion [5,9]. In addition, the synergistic interaction between SRB

and others bacteria will accelerate the metabolism activities due to formation of high biofilm product. Till date, study of this consortium bacteria especially in the present of SRB in tropical environment is still rare. In this paper, the effect of consortium bacteria containing SRB from tropical environment has been performed on carbon steel material. The analysis was carried out by weight loss and potentiodynamic polarization methods. Biofilm and morphology of carbon steel were investigated by variable pressure scanning electron microscopy (VPSEM) and energy dispersive X-ray spectroscopy (EDX).

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**Methodology:****Bacteria and culture condition:**

C-SRB used in this work was obtained from biological laboratory, Faculty of Science and Technology, UKM. This bacteria was isolated from local crude oil in Peninsular of Malaysia. Samples were collected in an anaerobic condition and followed the recommendations for SRB sampling. The bacteria were cultured and growth using VMNI medium as proposed by Zinkevich *et al.* [12]. The medium was prepared by using filtered seawater. The composition (g/L) of VMNI medium consists of 0.5 KH<sub>2</sub>PO<sub>4</sub>, 1.0 NH<sub>4</sub>Cl, 4.5 NaSO<sub>4</sub>, 0.3 sodium citrate, 0.04 CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.06 MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.0 casamino acids, 2.0 tryptone, 6.0 lactate, 0.1 ascorbic acids, 0.1 thioglycolic acids and 0.5 FeSO<sub>4</sub>·7H<sub>2</sub>O. The pH of the medium was adjusted in range of 7.0 to 7.2 using 1.0 M NaOH prior to autoclaving at 121 °C. 1.0 mL of trace element and 1.0 mL of vitamins were added after autoclaving process and the medium was left to cool down at room temperature before inoculating with C-SRB. Confirmation of presence SRB spesis in C-SRB was perform by SRB bar test kit.

**Weight loss test:**

The carbon steel coupon was mechanically cut to 12.0 mm diameter and 3.0 mm thickness. Chemical composition (wt%) of this steel was 0.12 C, 0.5 Mn, 0.045 S 0.04 P and balance is Fe. The coupon for weight loss test was ground with SiC grit paper grade 240, 320, 400, 600, 800 and 1200. At each grinding steps, the coupons were washed with distilled water and rinse with acetone. Total surface area and initial weight were determined. All coupons were immersed in 100 mL test bottle containing VMNI medium and 5 mL C-SRB batch. Sample with absense C-SRB was also prepared as a control. Weight loss test was carried out for a period of 1 to 15 days in incubator at 30°C. At each incubation periods, the coupon was withdrawn and cleaned according to ASTM G1-03. The Weight different was measured by analytical balance and the corrosion rate,  $C_R$  (mm/yr) was determined by equation 1.

$$C_R = \frac{\Delta WK}{At\rho} \text{ Corrosion rate,} \quad (1)$$

where  $K$  is conversion constant ( $3.45 \times 10^6$ ),  $\Delta W$  is weight loss (g),  $A$  is exposed area (cm<sup>2</sup>),  $t$  is the exposure time (h) and  $\rho$  is the density of sample coupon (g/cm<sup>3</sup>).

**Potentiodynamic polarization test:**

Coupon (as a working electrode) for potentiodynamic polarization test was embedded in resin epoxy except the working surface area. The working surface area and the sampel preparation were prepared as in weight loss procedure. The

medium was purged with oxygen-free nitrogen gas to create an anaerobic environment. Potentiodynamic polarization test was performed by using potentiostat model Gamry PC4/750 in 130 mL glass cell based on conventional three electrode. Graphite electrode and saturated calomel electrode were used as counter electrode and reference electrode, respectively. All test were done at temperature 25°C after 20 minutes exposure time. The potential was scanned in range of -250 to +250 mV at scan rate 1.0 mV/s.

**Colony forming unit:**

The growth of C-SRB from 1 to 15 days has been measured by using dilution and plate counting technique. From this technique, the number of C-SRB colony forming unit (CFU),  $N_{SRB}$  can easily determined as equation 2 [10];

$$N_{C-SRB} = \frac{N_c \times d_F \times 1 \text{ mL}}{0.1 \text{ mL}} \quad (2)$$

Where  $N_c$  is the number of colony count and  $d_F$  is the dilution factor.

**Surface and biofilm analysis:**

Surface analysis on the biofilm production and the effect of microbial corrosion had been perform by VPSEM coupled with EDX spectroscopy. The prepared coupon, which is inoculated with and without C-SRB were withdrawn and immersed in 2% of glutaraldehyde solution for 1 hour. Later, sample was gradually dehydrated in ethanol for 10 minute in each of 35%, 70%, 80%, 90% and 100% ethanol. Beside, sample for microbial corrosion was withdrawn and cleaned according to ASTM G1-03, before been washed with distilled water and rinse with acetone. All coupons were sputtered with gold prior to analysis.

**Results and Discussions****Isolated C-SRB growth:**

Figure 1 shows the curve of C-SRB growth in VMNI medium after incubated at 30°C for 1 to 15 days. The result indicated that the growing process of C-SRB can be divided into three stages which are exponential phase, stable state and decline period. The first stage was started from the day one until the day three. The number of SRB increased rapidly from day three to day seven and achieved the maximum at  $1.6 \times 10^{14}$  CFU. After the seventh day, the C-SRB growing process reached the second stage. From day nine to 11, the number of C-SRB maintained its growing process in approximately  $1.3 \times 10^{14}$  CFU. The last stage known as a dead phase. The curve shows that the number of SRB decreased rapidly and considered die. The growth of C-SRB at this certain period was due to the nutrient limitation in the medium.

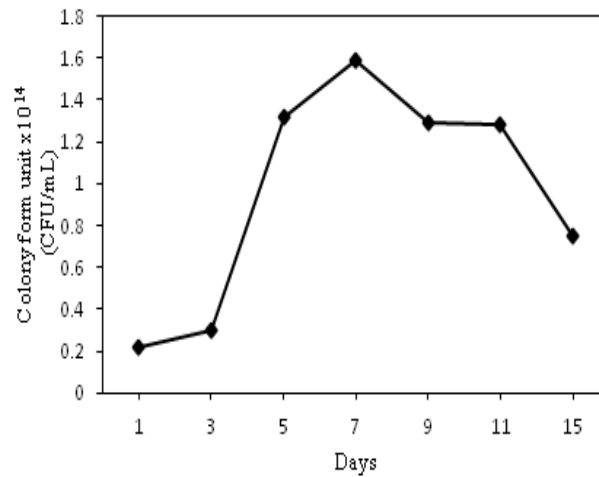
**The effects of corrosion by SRB:**

The corrosion rates of the carbon steel

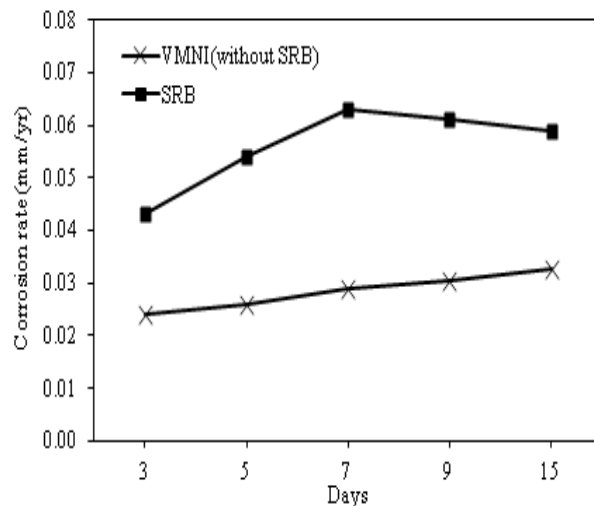
inoculated with and without C-SRB from weight loss method were shown in Figure 2. As can be seen, the corrosion rates of carbon steel exposed to C-SRB are higher than control medium at all growing stages. The maximum value, 0.063 mm/yr is obtained at day seven. This maximum value was in a good agreement with the CFU measurement as shown in Figure 1. It is suggested that the large number of density cell bacteria formed and C-SRB colony is capable of formatting a biofilm on the metal surface. The formation of this biofilm will accelerate the metabolism activity and the reduction of sulfate to sulfide. Furthermore, the formation of  $H_2S$  were taken part as an electrochemical product. This result is due to proven of rotten egg odor and observation of dark color of  $FeS$  as a product reaction between  $Fe$  ion and  $H_2S$  in VMNI medium. At this condition the microbial activities is maximum and account for

cause of microbial corrosion.

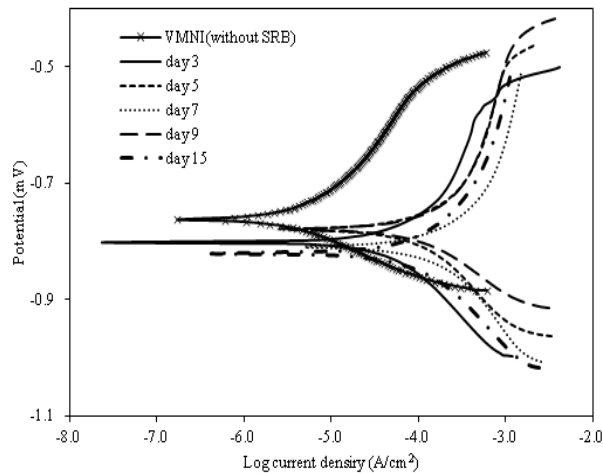
Figure 3 illustrates the polarization curves of carbon steel exposed to VMNI medium with and without C-SRB up to 15 days immersion time. All electrochemical parameters were calculated based on Tafel extrapolation and the data were tabulated in Table 1. The maximum value of corrosion rate from this analysis was also occurred on the seven days immersion. This result supports the obtained result of weight loss analysis, The highest corrosion rate in the presence of C-SRB reflected the biofilm formation with high rate of bacteria metabolism and caused a changing in electrochemical process. These conditions also influenced by the redox activities in VMNI medium, thus accelerated the carbon steel dissolutions [3,7,11].



**Fig. 1:** The growth curve for C-SRB in VMNI medium for 15 days incubation.



**Fig. 2:** Weight loss and corrosion rate of carbon steel in VMNI medium and C-SRB inoculated at different incubation period.

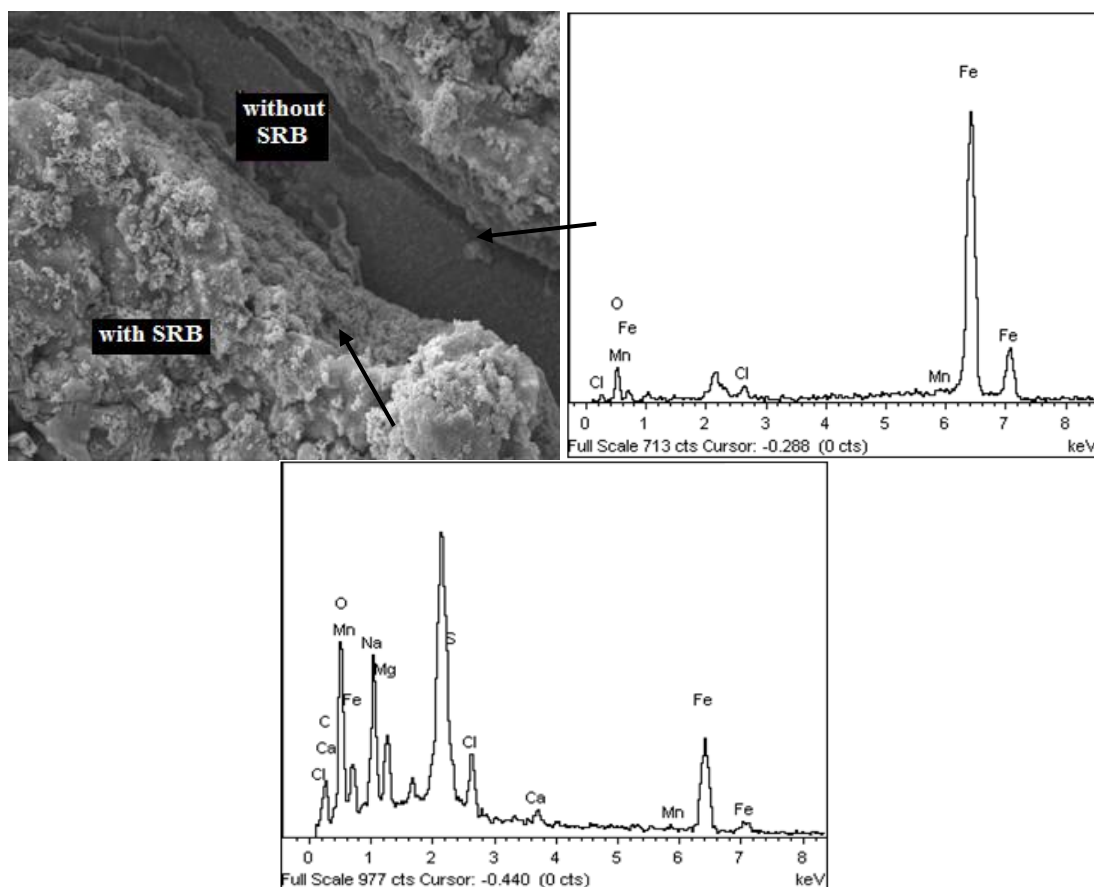


**Fig. 3:** Potentiodynamic polarization curve of carbon steel in VMNI medium and C-SRB inoculated at different incubation period.

*Surface morphology and elemental observations:*

The morphology of VPSEM image and quantitative EDX analysis of carbon steel immersed in absence and presence of C-SRB are shown in Figures 4 and 5. EDX analysis revealed that the corrosion products and biofilm formation were distributed over the coupon surface. As can be observed in Figure 4, a large amount of Ferum and Sulfur can be observed from these regions. The

presence of these two elements on carbon steel surface revealed that the formation of C-SRB metabolism occurred in this medium (Fonseca *et al.*, 1998). Figure 5(a) illustrates a rough surface of carbon steel without any microbial cell. However, in Figure 5(b), the C-SRB with high density of cell bacteria such as rod-shape, vibro and coccus were observed together with corrosion products and EPS.



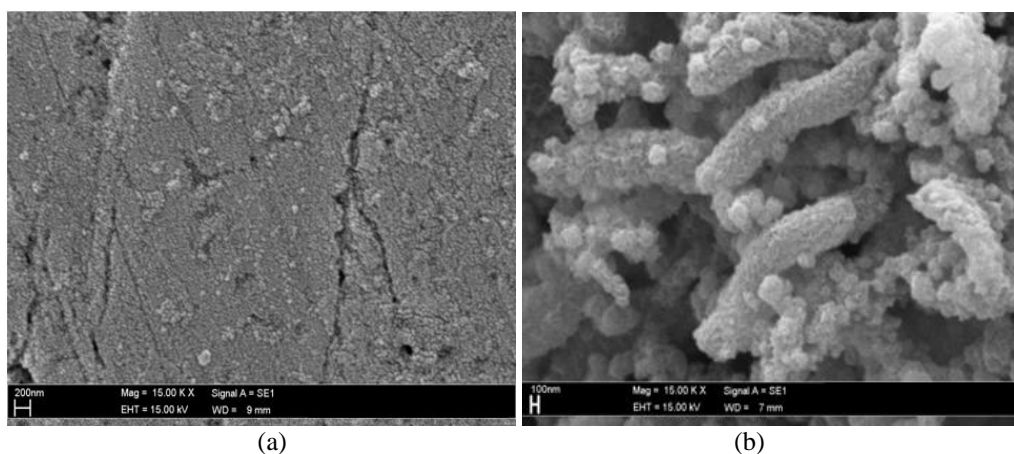
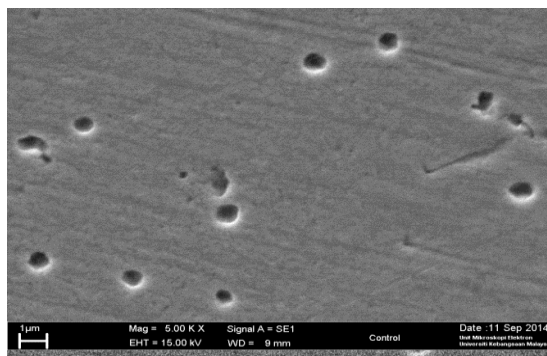
**Fig. 4:** VPSEM and EDX analysis of carbon steel exposed to VMNI medium inoculated with C-SRB.

**Table 1:** Result of potentiodynamic polarization for carbon steel in VMNI medium with and without C-SRB up to 15 days immersion time.

Sample (days)	$E_{corr}$ (mV)	$I_{corr}$ ( $A/cm^2 \times 10^{-4}$ )	$\beta_c$ (mV/Dec)	$\beta_a$ (mV/Dec)	Corrosion rate (mm/yr)
Without SRB	-653.2	0.98	114.7	143.2	0.58
3	-802.6	1.19	284.2	315.9	1.43
5	-779.8	4.72	437.9	745.6	5.65
7	-809.9	7.44	495.7	781.7	6.91
9	-780.0	3.40	226.8	593.4	4.08
15	-876.8	2.17	160.0	488.0	3.61

The obtained results indicate that, the formation of biofilm and attachment of SRB species on this metal surfaces were taken place at the early stage of this metabolism process. Further activities by this microbial had bring about formation of extracellular polymeric substances (EPS). Beech and Gaylarde (1999) in their study had quantitatively discovered

that EPS and corrosion products were occupied around 75-95% in total biofilms volume, while 5-25% was occupied by the cells. Morphology of carbon steel surface exposed to C-SRB for 15 days are presented in Figure 6. The results show that localized pitting corrosion occurred on this surface due to microbial activities.

**Fig. 5:** VPSEM image of carbon steel after 7 days exposed in (a) VMNI medium and (b) medium inoculated with C-SRB.**Fig. 6:** VPSEM image of typical pits on carbon steel surface exposed to C-SRB.

#### Conclusion:

C-SRB from tropical environment had effectively corroded the carbon steel surface through its natural metabolism process. All analysis show that the metabolism of C-SRB is optimum at seven days incubation period. Maximum growth of this consortium was  $1.6 \times 10^{14}$  CFU/mL with the highest corrosion rate at 0.063 mm/yr and 6.91 mm/yr from both weight loss and potentiodynamic polarization analyses, respectively. C-SRB activities on the carbon steel surface was also proven by morphological analysis. However, further study on

inhibiting this corrosion process and biocide effect on present C-SRB is still need to be considered for further investigation.

#### Acknowledgement

This work was partially supported by ERGS/1/2012/ST205/UKM/02/2 and FRGS/2/2013/SG06/UKM/02/4 grant. Nur Akma Mahat would like to thank the Ministry of Higher Education Malaysia for the MyBrain15 (My Master) scholarship.

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