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Inhibition of Consortium Sulfate Reducing Bacteria from Crude Oil for Carbon Steel Protection

(Perencatan Konsortium Bakteria Penurun Sulfat daripada Minyak Mentah sebagai Pelindung Keluli Karbon)

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ABSTRACT

The efficiency of cetyltrimethylammonium bromide (CTAB) to reduce the activity of consortium bacteria consisting of sulphate-reducing bacteria (C-SRB) has been investigated on variable concentration by weight loss test, potentiodynamic polarization and diffusion disk methods. C-SRB was isolated from tropical crude oil of Malaysian offshore. Biofilm analysis was also evaluated by variable pressure scanning electron microscopy (VPSEM). Weight loss and potentiodynamic polarization analyses showed that CTAB is able to inhibit the biocorrosion process and their inhibition efficiency had reached to 85 and 65% at 300 ppm CTAB, respectively. Increasing of CTAB efficiency as a function of concentration was also supported by diffusion disk analysis. Biofilm analysis showed that less of C-SRB and their metabolic by-product had been observed. It was concluded that CTAB was able to reduce the C-SRB activity and prevent biocorrosion process on carbon steel surface.

Keywords: Carbon steel; consortium SRB; potentiodynamic polarization

ABSTRAK

Kecekapan Cetriltrimetilammonium bromida (CTAB) untuk mengurangkan aktiviti bagi konsortium bakteria yang mengandungi bakteria penurun sulfat (C-SRB) telah dikaji pada kepekatan yang berbeza dengan menggunakan kaedah kehilangan berat, pengutuban potensiodinamik dan resapan cakera. SRB telah diasingkan daripada minyak mentah tropika luar pesisir Malaysia. Analisis biofilem juga dinilai dengan mikroskopi pengimbas elektron pemboleh ubah tekanan (VPSEM). Analisis kehilangan berat dan pengutuban potensiodinamik mendedahkan bahawa CTAB mampu untuk menghalang berlakunya proses biokakisan dan kecekapan perencat yang ditunjukkan bagi kedua-dua kaedah telah mencapai kepada 85 dan 65% pada 300 ppm CTAB. Peningkatan kecekapan CTAB sebagai fungsi kecekapan juga disokong oleh analisis resapan cakera. Analisis biofilem menunjukkan bahawa terdapatnya pengurangan daripada sel C-SRB dan juga hasil produk metabolik bakteria telah diperhatikan. Kesimpulannya, CTAB mampu untuk mengurangkan aktiviti C-SRB dan juga mencegah daripada berlakunya proses biokakisan terhadap keluli karbon.

Kata kunci: Keluli karbon; konsortium SRB; pengutuban potensiodinamik

INTRODUCTION

Microbiologically influenced corrosion (MIC) was recognized as an imperative factor for materials degradation especially in the petroleum industry. This insidious threat was impacting major failures in the transportation pipeline system due to biocorrosion menace. Bacteria adhesion and biofilm formation are always experienced both in the natural environment and industrial process. Metabolic by-products from these microbial was found to affect the kinetics of cathodic or anodic reaction and also modify the chemistry of protective layer thus accelerating the corrosion process (Javaherdashti 2011; Mansfeld 2007).

It was reported that 40% of the corrosion in interior pipelines gas was due to sulfate reducing bacteria (SRB) (Turkiewicz et al. 2013). SRB was categorized as anaerobic bacteria or in other words, do not require oxygen to grow and SRB derive their energy from organic nutrients. SRB can grow at pH4 to 9.5 and able to survive in temperature from 25 to 60°C (Alabbas et al. 2013). SRB has also been reported to cause contaminating of crude oil and increasing the sulfur level in petroleum oil. SRB reduces sulfate compounds to sulfide in their metabolic process. Further reaction of sulfide ions and hydrogen gas will contribute to the production of hydrogen sulfide (H_2S) which is harmful to the environment and most of metallic compound (Alabbas et al. 2013; Javaherdashti 2011). Thus, prevention of this menace acid is so essential.

Among the organic compounds, quaternary ammonium has been considered as an effective compound due to its capability to react as a bifunctional namely inhibitor as well as antimicrobial agents. Quaternary ammonium compounds are also known as cationic bioactive agents that have been extensively used in industrial, domestic, agricultural and clinical applications. In contrast to other antimicrobials, this compound are not chemically transform after application by retain their biocidal properties (Carmona-ribeiro et al. 2013). The effectiveness shown by quaternary ammonium compound is due to adsorption

properties on metal surface and for antimicrobial action is based on their ability to disrupt the metabolic activity and consequently caused the death of the bacteria (Shaban et al. 2013).

The objective of this study was to investigate the efficiency of CTAB on variable concentration, acting as a corrosion inhibitor and antimicrobial for carbon steel in medium containing C-SRB.

Materials and Methods

C-SRB used in this work was obtained from Biological Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia which was isolated from local crude oil from Malaysian offshore. C-SRB was cultured in anaerobic condition using VMNI medium as proposed by Zinkevich et al. (1996). The composition of VMNI medium is shown in Table 1. pH of this medium was adjusted in the range of 7.0 to 7.2 by using a few drops of 1.0 M NaOH prior to autoclaving at 121°C. Later, 1.0 mL of trace element and 2.0 mL of vitamins were added after autoclaving process and the medium was left to cool down at room temperature (Sahrani et al. 2009).

TABLE 1. Composition of 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 acids	0.1
$FeSO_4.7H_2O$	0.5

Batch sample of C-SRB was inoculated in VMNI medium by using 28 mL universal bottle. These samples were incubated for three days at 30°C and followed by centrifuging process at 1500 rpm for 5 min. New VMNI medium, CTAB concentration and carbon steel coupons were added based on the present analysis methods.

Chemical composition of low carbon steel employed in this study is 99.3 wt. % Fe, 0.12 wt. % C, 0.5 wt. % Mn, 0.04 wt. % P and 0.045 wt. % S. This carbon steel was mechanically cut to a coupon size and ground with abrasive paper from 240 to 1200 grit. At each of grinding steps, coupons were washed with distilled water and rinse with acetone.

Weight loss test was carried out by immersing carbon steel coupon in VMNI medium that inoculated with prepared C-SRB batch. CTAB concentration was varied at 100, 200 and 300 ppm. The samples were reincubated at 30°C for the 7 days incubation period. Later, carbon steel coupons were withdrawn and cleaned according to ASTM G1-03.

The potentiodynamic polarization test was performed by using a potentiostat model Gamry PC4/750 in three-electrode electrochemical cell. Graphite and saturated calomel electrode (SCE) were used as counter and reference electrodes, respectively. Working electrode was connected with a copper wire and embedded in epoxy resin. Potentiodynamic polarization test was carried up at 1.0 mV/s of scan rate within a potential range -250 to +250 mV of open circuit potential.

The diffusion disk method was performed in cell-culture dish that contain VMNI agar medium. A standard filter paper disk was dipped in different CTAB concentration. Cell-culture dish was swept with C-SRB and laid by standard filter paper disk prior to incubating at 30°C for 24 h. Later, diameter of inhibition zone had been measured.

Biofilm analysis was performed by VPSEM Model LEO 1450VP. The prepared samples were immersed in 2% of glutaraldehyde solution for 1 h prior to sequentially dehydrating ethanol concentration (35, 70, 80 and 100%) for 10 min, respectively. All samples were sputtered with gold.

Results and Discussion

The analysis of corrosion rate, *CR* (mm/yr) and inhibition efficiency, *IE%* obtained from the weight loss test are shown in Figure 1. These corrosion rate and inhibition efficiency were calculated based on (1) and (2), respectively:

$$CR = K\Delta W/(At\rho), \quad (1)$$

where K is the conversion constant (8.76×10^4), t is the exposure time (h), A is the exposed area (cm^2), ΔW is the average weight loss (g) and ϱ is the carbon steel density (g/cm^3).

$$IE\% = 100 \times (1 - CR_i/CR_o), \quad (2)$$

where CR_i and CR_0 are the corrosion rate of carbon steel in the presence and absence of inhibitors, respectively.

As clearly observed in Figure 1, the corrosion rate decreased with the increasing of CTAB concentration, suggesting that the corrosion rate of the present carbon steel is a function of CTAB concentration. The value of corrosion rate without inhibitor was 0.063 mm/yr. By adding CTAB, the corrosion rate had drastically decreased. It is shown that at 300 ppm of CTAB, the corrosion rate reached to 0.01 mm/yr. Consequently, at this concentration, the inhibition efficiency has increased to 85%. The influence of CTAB on protecting corrosion process showed that this inhibitor can react as a barrier on the carbon steel surface and minimized the dissolution of cationic from the metal surface (Shanthy et al. 2009).

Figure 2 shows the result of potentiodynamic polarization test, which was carried out in VMNI medium

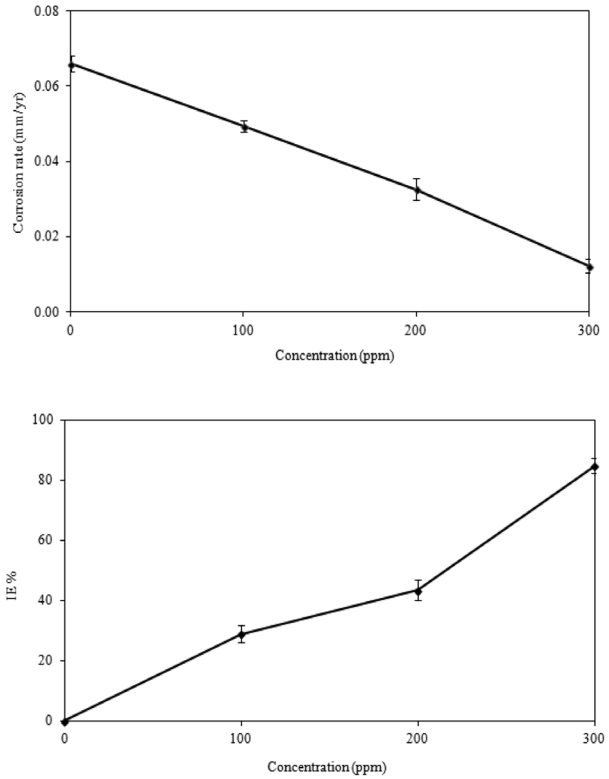


FIGURE 1. (a) Corrosion rate and (b) inhibition efficiency of carbon steel in various concentrations of CTAB

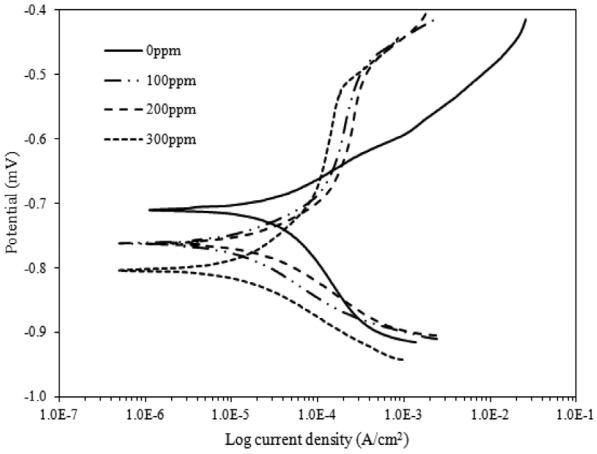


FIGURE 2. Tafel plot of consortium SRB and CTAB in VMNI medium for 7 days immersion

at the presence and absence of CTAB. The analysis was performed by Tafel extrapolation technique and detail electrochemical parameter, i.e. corrosion potential (E_{corr}), corrosion current density (i_{corr}), anadic and cathodic Tafel slopes (β_a and β_c) and corrosion rate (CR) are tabulated in Table 2. The result showed that the i_{corr} values decreased with the increasing of CTAB concentration. It is noticeable that the corrosion rate had also decreased as compared with control solution. The inhibition efficiency achieved at 63% by applying 300 ppm CTAB. It is suggested that CTAB is capable of protecting carbon steel in the C-SRB environment. As can be seen in Figure 2, the plot of all presences CTAB had slightly shifted to a cathodic region. Thus, all corrosion potential (E_{corr}) was shifted toward lower negative value as compared with free CTAB molecules (-709.2 mV). It is important to note here that CTAB molecules is able to protect the metal surface by shifted the corrosion mechanism toward noble process (Shanthy et al. 2009). The result from this analysis was in good agreement with the weight loss analysis.

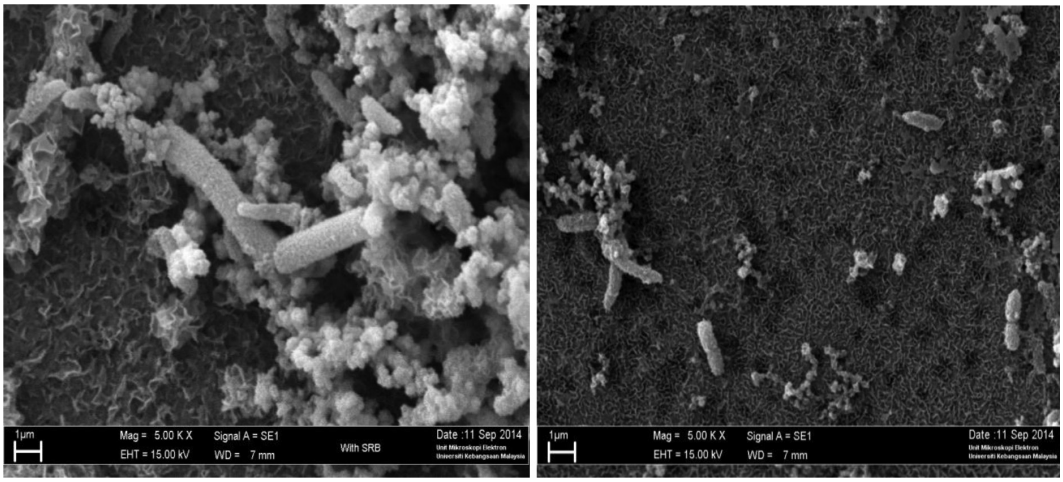
The efficiency of CTAB was evaluated by the existing of inhibition zone diameter. Figure 3 depicts the inhibition zone of different CTAB concentration on the cell-culture dish. It is calculated that the diameter of inhibition zone for 100, 200 and 300 ppm CTAB was 18, 22 and 24 mm, respectively. This value was gradually increased by the increasing of CTAB concentration and the maximum diameter was obtained at 300 ppm CTAB. This result indicated that the effectiveness of CTAB compound on microbiologically activity had been affected by absorption of CTAB compound toward negative charges of bacteria cellular membrane. Active reaction of CTAB molecules with the bacteria presence caused a decreasing in cell osmotic stability and leaking the intracellular constituents (Negm et al. 2011; Rabea et al. 2003).

The influence of CTAB on biofilm product had been analyzed by VPSEM and their micrographs are shown in Figure 4(a) and 4(b). Micrograph in Figure 4(a) depicted a distribution of C-SRB, extracellular polymeric substances (EPS) and the corrosion products. It is clearly seen that a rod shape bacteria was seemly show a feature of *Disulfovibrio* cell that is belong to SRB species. However, this genetic sequence has not been studied yet and will be carried out in future work. EPS compound, which is arising from C-SRB metabolic process was normally, consists of polysaccharides, lipids, protein and nucleic

TABLE 2. Result of Tafel polarization test for carbon steel in VMNI medium with consortium SRB and CTAB for 7 days immersion

Concentration CTAB (ppm)	E_{corr} (mV)	I_{corr} (μA/cm²)	β_c (mV/Dec)	β_a (mV/Dec)	Cr (mmy)	η %
0	-709.2	0.45	208.4	98.2	0.526	-
100	-762.7	0.34	154.1	245.2	0.401	24
200	-760.8	0.28	100.5	114.4	0.326	38
300	-802.6	0.17	88.9	149.4	0.196	63

FIGURE 3. Diffusion disc shows the inhibition zone diameter of CTAB at different concentration



(a) (b)

FIGURE 4. VPSEM analysis for carbon steel surface exposed to the C-SRB cultured for 72 h of immersion in VMNI (a) without CTAB (b) and with CTAB

acids. These presence compounds can create a protective shield and behave as a barrier between carbon steel and its environment (Flemming & Wingender 2010; Videla & Herrera 2009). However, in Figure 4(b), fewer amounts of bacteria cells and EPS formation have been presented. This analysis indicated that CTAB molecules are able to retard the growth of C-SRB and minimized the metabolic process. It is suggests that CTAB can be considered as a potential compound to inhibit the C-SRB activities and protect the carbon steel from continuously degrade.

CONCLUSION

The effect of CTAB as an inhibitor on carbon steel and antimicrobial for C-SRB in tropical environment was investigated. As a conclusion, the higher the concentration of CTAB, the higher the inhibition efficiency of carbon steel and the higher the diameter of inhibition zone were shown as antimicrobial against C-SRB.

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REFERENCES

Alabbas, F.M., Bhola, R., Spear, J.R., Olson, D.L. & Mishra, B. 2013. Electrochemical characterization of microbiologically influenced corrosion on linepipe steel exposed to facultative anaerobic desulfovibrio sp. *Int. J. Electrochem. Sci.* 8: 859-871.

Carmona-ribeiro, A.M., Dias, L. & Carrasco, D.M. 2013. Cationic antimicrobial polymers and their assemblies. *Int. J. Mol. Sci.* 14: 9906-9946.

Flemming, H-C. & Wingender, J. 2010. The biofilm matrix. *Nature Reviews Microbiology* 8: 623-633.

Javaherdashti, R. 2011. Impact of sulphate-reducing bacteria on the performance of engineering materials. *Appl. Microbiol. Biotechnol.* 91: 1507-1517.

Mansfeld, F. 2007. The interaction of bacteria and metal surfaces. *Electrochim. Acta* 52: 7670-7680.

Negm, N.A., Ghuiba, F.M., Mahmoud, S.A. & Tawfik, S.M. 2011. Biocidal and anti-corrosive activities of benzoimidazol-3-ium cationic Schiff base surfactants. *Eng. Life Sci.* 11: 496-510.

Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G. & Steurbaut, W. 2003. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* 4: 1457-1465.

Shaban, S.M., Saied, A., Tawfik, S.M., Abd-Elaal, A. & Aiad, I. 2013. Corrosion inhibition and Biocidal effect of some cationic surfactants based on Schiff base. *J. Ind. Eng. Chem.* 19(6): 2004-2009.

Shanthy, P., Rengan, P., Chelvan, A.T., Rathika, K. & Rajendran, S. 2009. Corrosion inhibition and biocidal activity of a cationic surfactant. *Indian J. Chem. Technol.* 16: 328-333.

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

Turkiewicz, A., Brzeszcz, J. & Kapusta, P. 2013. The application of biocides in the oil and gas industry. *Nafta-Gaz* R69(2): 103-111.

Videla, H.A. & Herrera, L.K. 2009. Understanding microbial corrosion inhibition: A comprehensive overview. *Int. Biodet. Biodeg.* 63: 896-900.

Zinkevich, V., Bogdarina, I., Kang, H., Hill, M.A.W., Tapper, R. & Beech, I.B. 1996. Characterisation of exopolymers produced by different isolates of marine sulphate-reducing bacteria. *Int. Biodet. Biodeg.* 37: 163-172.

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