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Influence of Benzyltriethylammonium Chloride on Biocorrosion Activity of Consortium Bacteria from Tropical Crude Oil

(Pengaruh Benziltrietilamonium Klorida terhadap Aktiviti Biokakisan Konsortium Bakteria daripada Minyak Mentah Tropika)

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ABSTRACT

The performance of pipeline system used in petroleum industry is crucially declined by natural microbial activities and demanding extra operational cost. Requirement on high capability of functional substances is attracting worldwide research interest. The aim of this paper was to study the effectiveness of benzyltriethylammonium chloride (BTC) on reducing the activity of a consortium bacteria consisting of sulfate-reducing bacteria (C-SRB). C-SRB was isolated from tropical crude oil and enumeration of this consortium was measured by viable cell count technique. The effectiveness of BTC was calculated from potentiodynamic polarization method and biofilm analysis was performed by scanning electron microscope. The viable cell count technique indicated that the maximum growth of C-SRB was approximately 160 trillion CFU/mL at 7 days incubation period. BTC was capable of reducing biocorrosion activity due to adsorption process and mitigating SRB species. Biofilm analysis has proven that C-SRB activity is minimized due to less presence of bacterial growth, extracellular polymeric substances and corrosion product. In conclusion, BTC is capable to inhibit C-SRB activity on biocorrosion of carbon steel pipeline.

Keywords: Carbon steel; consortium bacteria; potentiodynamic polarization

ABSTRAK

Keupayaan sistem saluran paip yang digunakan dalam industri petroleum banyak mengalami penyusutan akibat aktiviti mikrob dan memerlukan kos pengoperasian yang tinggi. Keperluan keupayaan yang tinggi daripada sebatian berfungsi telah menarik minat penyelidikan seluruh dunia. Matlamat kajian ini adalah untuk mengkaji kecekapan benziltrietilamonium klorida (BTC) terhadap penurunan aktiviti konsortium bakteria yang mengandungi bakteria penurun sulfat (C-SRB). C-SRB telah dipencilkan daripada minyak mentah tropika dan pengangkaan konsortium ini telah dihitung melalui teknik kiraan sel boleh hidup. Kecekapan BTC ditentukan daripada kaedah kekutuban keupayaan dinamik dan analisis biofilem telah dijalankan dengan menggunakan mikroskop elektron imbasan. Teknik kiraan sel boleh hidup mendapati nilai pertumbuhan maksimum C-SRB dianggarkan berjumlah 160 trillion CFU/mL dalam tempoh 7 hari pengeraman. BTC didapati berupaya mengurangkan aktiviti biokakisan melalui proses penjerapan dan pengurangan spesies SRB. Analisis biofilem membuktikan bahawa aktiviti C-SRB adalah minimum dengan penggunaan BTC disebabkan kurangnya pertumbuhan bakteria, juga sebatian polimer ekstrasel dan hasil kakisan. Sebagai kesimpulan, BTC didapati berupaya menyekat aktiviti C-SRB dan proses biokakisan pada permukaan paip keluli karbon.

Kata kunci: Kekutuban keupayaan dinamik; keluli karbon; konsortium bakteria

INTRODUCTION

Pipeline is a common transportation system used to transfer crude oil and other liquid petroleum product. However, microbiologically induced corrosion is a common cause that reduced the performance of this pipeline (Al-Jaroudi et al. 2011). The serious threat normally comes from sulfate-reducing bacteria (SRB) (Kakooei et al. 2012). SRB used sulfate as their electron acceptor and reduce this compound to sulfide ions. The reaction between these ions and hydrogen increases the amount of H_2S in crude oil medium and lead the corrosion process. As a result, the formation of iron sulfide as a corrosive product will be deposited (Sahrani et al. 2008b). The ubiquity of these bacteria as a consortium with different groups of microorganism will promote large amount of H_2S and metabolic by-product.

In order to minimize this insidious threat, several actions such as monitoring the biocontamination of oil field units, mapping regional biocontamination, monitoring at suppression of microbial growth and using appropriate biocides should be performed (Moiseeva & Kondrova 2005). Among these, biocide injection based on organic compounds such as chlorine-releasing agents, quaternary ammonium compounds, glutaraldehyde, tetrakis (hydroxymethyl) phosphonium sulfate and phenolic compounds has been widely used in industry particularly in pipeline system (Ortega Morente et al.

2013). The demand on high capability of biocide materials is still attracting worldwide research.

However, study on benzyl triethylammonium chloride (BTC) as a bifunctional material namely as corrosion inhibitor as well as to control consortium bacteria population has not been fully explored yet. Benzyl group in BTC is expected to have the ability to poison the consortium bacteria. The objective of this study was to determine the effectiveness of BTC on reducing C-SRB activities and preventing biocorrosion menace on carbon steel surface at highest corrosive environment arise from this consortium. Here, the growth of C-SRB in VMNI medium had been enumerated by viable cell count method and the efficiency of BTC has been investigated by potentiodynamic polarization method and scanning electron microscopy.

Materials and Methods

A commercial grade carbon steel API 5L pipe with chemical composition of 0.258 wt. % C, 0.466 wt. % Mn, 0.427 wt. % Si, 0.013 wt. % P, 0.015 wt. % Ni, 0.019 wt. % Cu, 0.132 wt. % Al and balance Fe was employed in this study. The carbon steel was cut to a coupon size of 12 × 10 × 5 mm. Carbon steel that was used as a working electrode was connected with a copper wire. The whole area of this working electrode was embedded in epoxy resin except the test surface. This surface was ground with emery paper up to 1200 grit, washed in distilled water and rinsed with acetone. BTC was purchased from Sigma Aldrich Co. Ltd. BTC concentrations were varied from 128 to 8192 ppm based on double dilution method.

C-SRB 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 grown using VMNI medium. VMNI medium was prepared from filtered sea water and added with (g/L) 0.5 KH_2PO_4, 1.0 NH_4Cl, 4.5 Na_2SO_4, 0.3 $Na_3C_6H_5O_7$, 0.04 $CaCl_2.6H_2O$, 0.06 $MgSO_4.7H_2O$, 2.0 casamino acid, 2.0 tryptone, 6.0 sodium lactate, 0.1 ascorbic acid, 0.1 thioglycolic acid and 0.5 $FeSO_4.7H_2O$ (Sahrani et al. 2008a). The pH of VMNI medium was adjusted in range of 7.0 to 7.2 using a few drops of 1.0 M NaOH solution. This medium was filtered sequentially with 0.45 μm and 0.22 μm mixed cellulose ester membranes. Solid VMNI medium was also prepared by adding 2% of agar. VMNI was autoclaved at 121°C for 15 min prior to addition of 0.1 mL trace element and 0.2 mL vitamins at room temperature.

C-SRB Batches were prepared by adding 5 mL of this consortium into 28 mL universal bottles containing VMNI medium. These samples were incubated for three days at 30°C prior to centrifuge at 3500 rpm for 5 min. These concentrated sediments had been used as C-SRB batches for further analysis. Viable cell count method was carried out by adding new VMNI medium to each of these C-SRB batches and all samples were reincubated at same temperature for 1 to 15 days. Enumeration growth of this consortium was determined by using solid VMNI medium in cell-culture dish.

The effectiveness of BTC on biocorrosion activity was studied by potentiodynamic polarization method. In this study, C-SRB batches obtained from previous method were added with new VMNI medium containing different BTC concentration. All samples were prepared in 100 mL testing bottle. The samples of working electrode (carbon steel) were immersed entirely in this medium prior to reincubated for 7 days at 30°C. Potentiodynamic polarization method was performed after this incubation period by means of a potensiostat, model Gamry PC4/750, using a conventional three electrodes cell. Saturated calomel electrode and platinum rod were used as reference and auxiliary electrodes, respectively. This analysis has begun after 20 min exposure time at room temperature. The potential was scanned between -250 to +250 mV about the open circuit potential and the scanned rate was set at 1.0 mV/s.

Biofilm analysis on carbon steel surface of presence and absence of C-SRB were analyzed by a scanning electron microscope, model Zeiss Supra 35VP. In this study, carbon steel coupons that were ground up to 1200 grit were immersed entirely in VMNI medium containing C-SRB batch with absence and presence of 4096 ppm BTC. These samples were also incubated for 7 days at 30°C. Later, coupons were withdrawn, dipped with double distilled water for 2 s and dried with normal air. The analysis was performed without any sputtering.

Results and Discussion

Viable cell count was used to determine the number of bacteria on culture medium. Figure 1 point out the bacterial colonies grew up from first to 15 days incubation period. The growth of colonies in this graph can be divided into three phases. At the early stage, it was clearly illustrated as an exponential phase, which has started from the first to 6 days of incubation. The number of colony increased rapidly from 25 to 140 trillion CFU/mL. The exponential phase in Figure 1 indicated that SRB secured thorough nutrient for growth and executed their metabolic process. At this stage, the grown SRB enhanced their inherent respiration process and produced a by-product component. During respiration process, SRB used sulfate and others sulfur compounds from VMNI medium as electron acceptor and reduce these compounds to sulfide ions. Reduction of sulfate compound and producing of metabolic by-product by SRB can be expressed as follows:

$$SO_4^{2-} + 8H \rightarrow S^{2-} + 4H_2O. \tag{1}$$

$$H^+ + S^{2-} \rightarrow H_2S. \tag{2}$$

$$Fe^{2+} + S^{2-} \rightarrow FeS. \tag{3}$$

Equation 1 indicated that SRB consume hydrogen to obtain the electron required by an enzyme known as hydrogenase (Alabbas et al. 2012). Consequently, these sulfide ions accelerated the formation of hydrogen sulfide

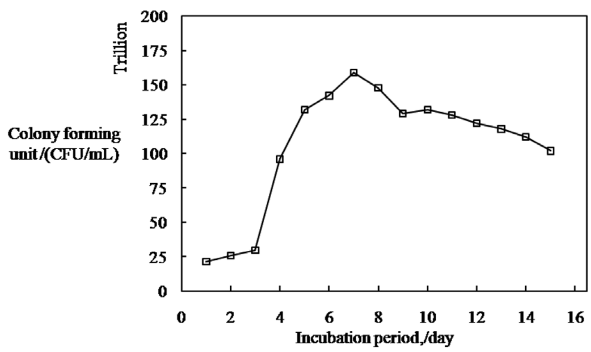


FIGURE 1. Number of colony growth at different incubation period

(H_2S) and iron sulfide (FeS) (Al-Jaroudi et al. 2011) as mention in (2) and (3), respectively. The presences of H_2S and FeS in this medium were detected by smelling of rotten egg odor and dark color of broth samples. This observation is a common practice used in detecting the production of H_2S and FeS arise from SRB activities (Abdullah et al. 2014; Li et al. 2009).

Second phase region began after 6 to 8 days of incubation period, where the number of bacteria grew steadily until reach a maximum value. It was noticed that the highest colonies emerged at 7 days incubation period at approximately 160 trillion CFU/mL. At this incubation period, it is believed that the formation of H_2S has reached the highest concentration thus providing crucial corrosive environment among other incubation periods. This incubation period has been selected as a main criterion in this study. However, the third phase of this plot can be considered as a dead phase. The amount of cells started to die after 9 days incubation and continuously declined as the incubation period increased. This was due to fewer amounts of nutrient sources for these SRB species to growth.

The performance of BTC for protecting carbon steel in the presence of SRB species (i.e. at highest corrosive environment) was carried out by potentiodynamic polarization method. Figure 2 represents some plots of this analysis at different BTC concentration after 7 days incubation period at 30°C. Tafel extrapolating technique has been applied to analyze the plots and the corrosion rate (C_r) was calculated using (4) (Mohamad et al. 2014):

$$C_r \text{ (mm/yr)} = (I_{corr} \text{ x } K \text{ x } EW)/(dA) \quad (4)$$

where I_{corr} is the corrosion current density (mA); K is a constant (3272); EW is the equivalent weight (g/equivalent); d is the density and; A is the surface area of working electrode.

Details of these electrochemical data was presented in Table 1. As compared with free-BTC, all curves shifted towards lower current density-axis. The shifted values of all corrosion current density are tabulated in the range of 0.004 to 0.016 mA/cm^2 as compared to 0.744 mA/cm^2 at absence of BTC. As shown in Table 1, the presence of C-SRB at free-BTC concentration indicating that the corrosion rate value 8.91 mm/yr, which is considered the worst case for carbon steel in anaerobic environment. This corrosion rate value proved that higher concentration of H_2S has been produced by SRB as evident in viable cell count analysis.

Electrochemical data indicated that BTC is capable of protecting carbon steel from biocorrosion reaction due to this C-SRB activity. As illustrated in Table 1, the corrosion rates slightly increased with the increasing of BTC concentration up to 1024 ppm and this result merely remain unchanged after using 2046 ppm. It was shown that the effectiveness of BTC slightly decreased by applying its high concentration. The effect of BTC reached almost plateau at concentration 2048, 4096 and 8192 ppm with the corrosion rate approximately 0.10 mm/yr. Increasing in these corrosion rate values with the increment of BTC concentrations were attributable to the presence of chloride ion in BTC compound itself. At the early stage, the increasing of chloride ion in the medium has led the electrostatic interaction among these negative charges and carbon steel surface (Dkhireche et al. 2013; Negm et al. 2012). The presence of high concentration of chloride ion was attracting more Fe atoms to dissolve into the medium prior to adsorption process of BTC molecules to take place by substitution process and create a thin film barrier (Idris et al. 2015). However, these corrosion rates values are considering acceptable for carbon steel to be used in anaerobic condition (Ahmad 2006).

TABLE 1. Potentiodynamic polarization parameter of carbon steel in VMNI medium containing C-SRB at different BTC concentration

BTC Conc. (ppm)	E_c (V)	I_c (mA/cm^2)	(-)B_c (V/dec)	B_a (V/dec)	C_R (mm/yr)
0	-0.81	0.744	0.496	0.782	8.91
128	-0.79	0.004	0.083	0.121	0.05
256	-0.86	0.006	0.070	0.308	0.07
512	-0.79	0.005	0.101	0.276	0.06
1024	-0.72	0.016	0.118	0.105	0.19
2048	-0.78	0.008	0.129	0.316	0.10
4096	-0.83	0.007	0.119	0.325	0.09
8192	-0.88	0.008	0.166	0.403	0.10

It is also observed in Figure 2 that all curves shifted to both anodic and cathodic regions, suggesting that BTC molecules actively affected by adsorption onto anodic dissolution region and controlled hydrogen evaluation process at cathodic region. It is noticeable from Table 1 that the values of all corrosion potentials (E_{corr}) are below than 0.085 V as compared with absence BTC. It is reported that if the displacement of E_{corr} is > 0.085 V with respect to control E_{corr}, the inhibitor can be seen as an anodic or cathodic type, whereas if the displacement < 0.085 V, the inhibitor can be classified as mixed type (Al-Amiery et al. 2014; Hegazy et al. 2014). This result suggested that BTC had acted as a mixed type corrosion inhibitor for carbon steel in this corrosive medium.

BTC is a polar molecule contains N atom with a positive charge and a benzyl functional group that contributing π-electrons for electrostatic reaction to take place and increase in delocalization (Yadav et al. 2013). These features provide a better probability for BTC to attract and adsorb onto carbon steel surface. Due to electrostatic attraction among positively charges from BTC molecules and cathodic sites of carbon steel, the adsorption process of BTC molecules absolutely reduce the evolution of hydrogen, thus protecting the cathodic part. Additional of benzyl group with the presence of π-electron has also contributing to delocalization process. In this process, π-electrons were easily translated to Fe atoms by adsorbing the surface and decrease the anodic current (Ahamad et al. 2012; Musa et al. 2012). Increasing of BTC molecules enhanced the adsorption process resulting in the steel surface was covered with a thin film layer. This result was in good agreement with our previous study on BTC in acidic medium based on weight loss method (Idris et al. 2013). The thin film has reacted as a barrier to protect the surface and avoid dissolution of atom from metal surface (Al-Sabagh et al. 2011). In the absence of BTC, it is believed that corrosive species will easily react with carbon steel and enhanced corrosion current density.

Scanning electron microscope has also supported the existing of SRB and their biofilm product as well as corrosion product. Figure 3 depicts the micrograph of existing C-SRB and biofilm products on carbon steel coupon. Figure 3(a) is related with the outcome of viable cell count, where some feature of the bacteria is similar with the *Desulfovibrio* species. However, this genetic sequence has not been performed in this study and will be undertaken in future work. Moreover, the corrosion products as well as extracellular polymeric substance (EPS) were also clearly observed in this micrograph. EPS is a metabolic by-product, which is produced by SRB together with the reduction of sulfate compound to sulfide ions (Mahat et al. 2015). EPS normally consist of polysaccharide components, lipid and protein (Sheng et al. 2010). The existing of bulk EPS proven that highest concentration of H_2S has been generated in this incubation period. It is clearly seen in Figure 3(b) that the presence of BTC at 4096 ppm has affected the growth of C-SRB and prevented the carbon steel from actively react with corrosive medium arising from the presence of H_2S. This BTC concentration has been selected based on the potentiodynamic polarization result, which is considered as almost consistent in its corrosion rate value. This micrograph shows less production of biofilm and corrosion products after 7 days of incubation period.

From these analyses, it was suggested that BTC is also able to control the corrosion reaction by mitigating reactive species through an electrostatic interaction among BTC and SRB species. The different of charges between ammonium compound and negative charges at centre of SRB cellular cell attracted BTC molecules to come closer and adsorb onto the cells. This interaction has disrupted the metabolic process within cytoplasm and definitely damaged the functional permeability membrane (Badawi et al. 2010). Besides that, as a group of cationic, BTC are also able to

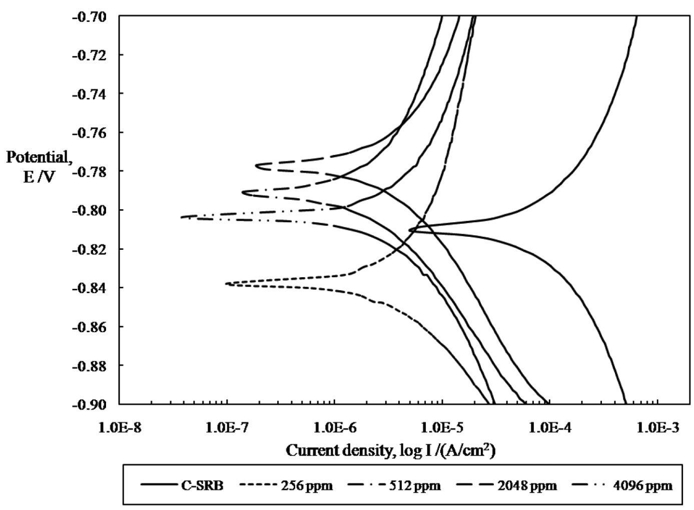
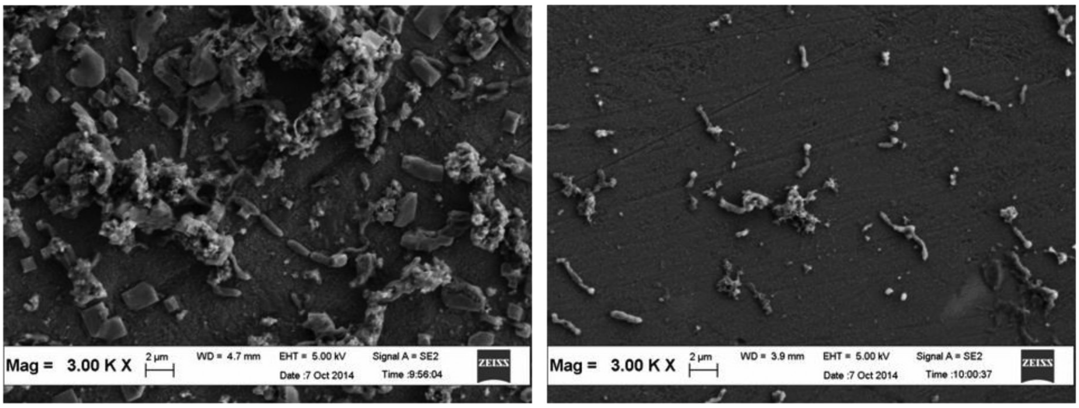


FIGURE 2. Potentiodynamic polarization plot of carbon steel in VMNI medium with C-SRB and different BTC concentration used



(a) (b)

FIGURE 3. SEM analysis on carbon steel pipe affected by consortium bacteria at (a) Free-BTC and (b) with 4096 ppm BTC

penetrate through the cell membrane and the positive charges of this compound is also potentially neutralized the negative charges on SRB cell membrane thus deactivated the selective permeability of outer cellular SRB membrane (Aiad et al. 2014). Consequently, the growth of SRB species in this consortium has been inhibit ascribed to death of SRB species and so, less amount of H_2S concentration has been generated due to less reduction of sulfate component as well as metabolic activity. However, further study should be carried out in order to understand its antimicrobial interaction through a biological approach such as diffusion disc method and biocidal activity.

CONCLUSION

This study showed that BTC is capable of controlling the growth of C-SRB and inhibit the biocorrosion process on carbon steel pipe. The highest corrosive environment arise from this C-SRB had gained after 7 days incubation period in VMNI medium at 30°C. The effectiveness of BTC in inhibiting biocorrosion process at this corrosive environment had slightly decreased with increasing of its concentration but it seen almost plateau at 0.10 mm/yr with the used of concentration at 2048, 4096 and 8192 ppm, respectively. It is also proven that BTC inhibit the biocorrosion of carbon steel by both adsorption and mitigation of reactive SRB species.

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