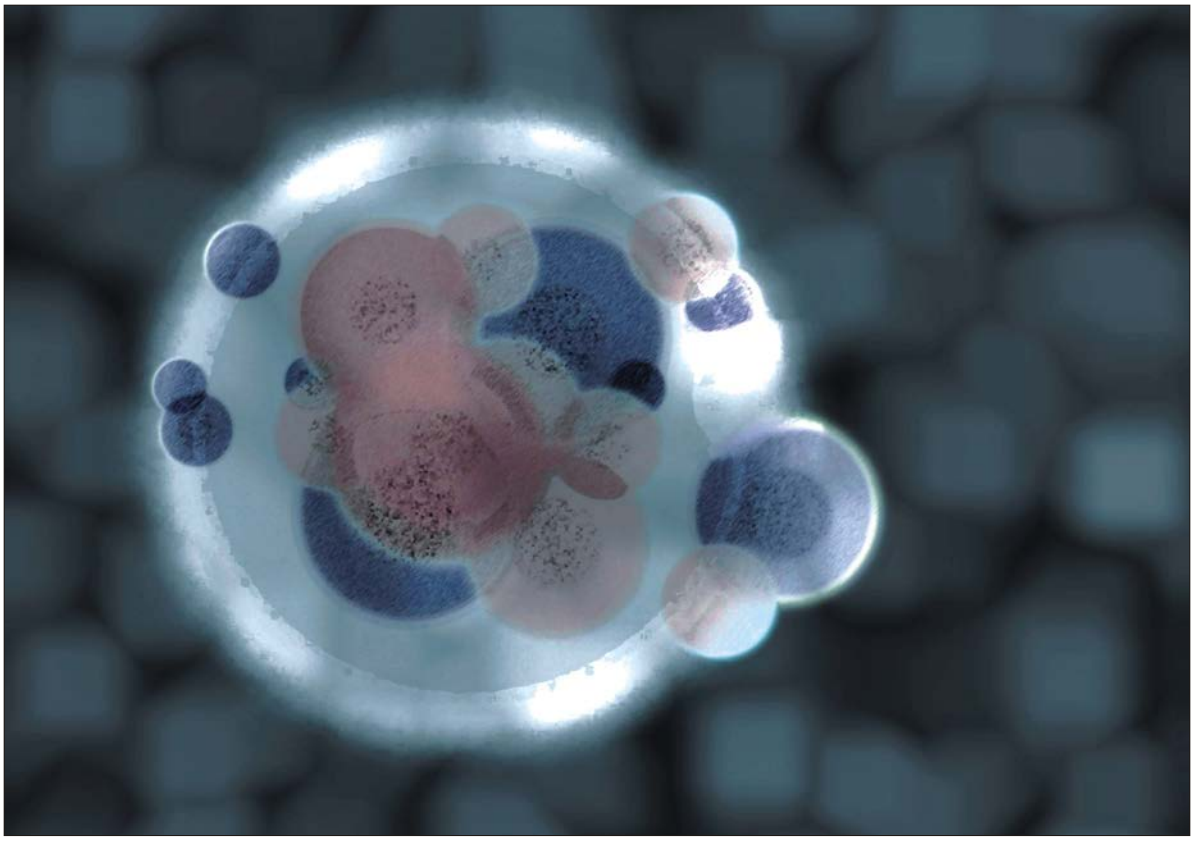

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Disinfection of Sulfate Reducing Bacteria using Ultraviolet Treatment in Mitigating Microbiologically Influenced Corrosion

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Abstract: Oil and gas pipeline systems often fail due to Microbiologically Influenced Corrosion (MIC). Chemical biocide is one of the common treatments to combat MIC. However, this method is costly and further usage of chemical biocides may lead to serious environment pollutions. In an attempt to disinfect *Desulfovibrio vulgaris*, a species involved in MIC from oil and gas pipelines, ultraviolet wave is used as a potential alternative. *D. vulgaris* (ATCC7757) was cultured in broth number 1249 (Modified Barr's Medium) and the medium was adjusted to pH 9.5 at 37°C based on the Sulfate Reducing Bacteria (SRB) optimum growth parameters. Carbon steel coupons grade API 5L X70 were cut to approximately 10×20×5 mm and kept in anaerobic vials along with the cultured medium. The prepared samples were incubated for 11 days, followed by exposure to ultraviolet for 1 h. Results from the study elucidated that ultraviolet (UV) is a viable option for SRB disinfection and could effectively reduce the growth of SRB.

Key words: Sulfate-reducing bacteria, corrosion, planktonic SRB

INTRODUCTION

Corrosion is one of the natural deterioration phenomenon that will affect financial and safety implications in oil and gas industry. Pipeline systems that are used to convey crude oils over long distances have always been facing serious corrosion problems that can lead to system failures (Noor *et al*., 2007). The failure investigation results indicated that the pipes have most likely suffered from severe corrosion damage due to Microbiologically Influenced Corrosion (MIC) (Al-Sulaiman *et al*., 2010). Microbial activity in any environment occurs in the presence of water, a carbon source, an electron donor and an electron acceptor, all of which can be present in oil pipelines (Almahamedh *et al*., 2011).

MIC is well known as one of the major causes that will accelerate the process of corrosion in pipelines. It implies that the increase in corrosion rate is due to the presence of bacterial activities that accelerates the rate of anodic and/or cathodic reactions (Mansfeld, 2007). One of the bacterial types that can be mostly found living in the absence of oxygen in the oil and gas pipelines is Sulfate Reducing Bacteria (SRB) or to be specific the species known as *Desulfovibrio vulgaris*. SRB are obligate anaerobes that obtain energy for growth from the oxidation of organic substances, using sulfate as the external electron acceptor (Widdle, 1988; Postgate, 1984). In oilfield systems, SRB can cause corrosion of iron in the absence of air and contamination of fuel gas and stored fuel gas with hydrogen sulfide (H_2S) (Cord-Ruwisch *et al*., 1987). Crudes containing H_2S are termed as sour crude and this can promote the growth of SRB. SRBs are anaerobic and do not need oxygen to survive and they use sulfate ions as a terminal acceptor and produce H_2S (Alabbas *et al*., 2012).

Biocides injection is the typical technique to mitigate MIC which is toxic and must be carefully handled cost ineffective and could cause environment pollutions. In some countries, the use of biocides has to follow stringent regulations. In order to minimise the use of biocides, ultraviolet technique has been introduced to mitigate MIC. Ultraviolet is an established technology for disinfection and controlling the bacterial populations. But the previous studies still lack optimization of the ultraviolet dosage according to specific bacterial properties and parameters. According to Barnard and Morgan (1903), the UV-radiation kills the microbial cells primarily due to its action on deoxyribonucleic acid (DNA). Bacteria, phages and viruses are also killed or reduced by photochemical wet combustion down to or below detection limits of organic carbon.

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The study presented explores the potential of ultraviolet as an inhibitor to SRB growth. *D. vulgaris* was cultured in a Modified Barr's Medium and samples were treated for 1 h and left incubated for 11 days. During the treatment process, samples were retrieved on several days to measure treatment efficiency. Field Emission Scanning Electron Microscopy (FESEM) was performed on the retrieved coupon before and after being exposed to SRB, to observe the effect of SRB on the carbon steel surface microstructure.

MATERIALS AND METHODS

Microorganism and media: The microorganism used in this study was SRB strain, *Desulfovibrio vulgaris* ATCC 7757 and cultured in a defined modified ATCC 1249 medium. Table 1 listed the composition of the modified ATCC 1249 medium. Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2$) at 25 ppm concentration was filtered and was add-in into the sterile medium.

Materials: The material used in this study is a carbon steel coupon grade API 5L X-70. Samples were cut into 10×20×5 mm dimension to fit the anaerobic vials openings. The test coupons were polished with 100 grit Si-C paper, cleaned and dried with ethanol to remove all form of dirt, grease and small Si-C particles on the coupon surface. The cleaned and dried coupons were then coated with prime coat leaving only the top surface exposed. The coupons were then dried overnight in an oven at 37°C. The exposed area of the coupon was polished again with series of SI-C paper grade (320, 600 and 800), followed by ethanol degreasing.

Method of inoculation: All of the medium compositions were mixed with 1 L of distilled water. The pH was adjusted by using buffer chemical to 7.0 and the test medium was sterilized in an autoclaved at 121°C for about 20 min. After cooling, the medium was purged with oxygen-free nitrogen for 1 h in a laminar flow hood to remove the oxygen in the solution. The test coupons and 100 mL of medium were then transferred into the 125 mL of anaerobic and sealed with butyl rubber to maintain the anaerobic condition. Two milliliter of 1 day old SRB seed was inoculated into the medium and incubated at 37°C. The black colour of the medium is the indicator of successful inoculation within 2-3 days. FESEM on the coupons before and after being exposed to SRB was performed to observe the effect of SRB to the carbon steel surface microstructure.

Table 1: Composition of the modified ATCC 1249 medium

Chemicals compositions	Values
Component 1	
$MgSO_4$ (g)	2.0
Sodium citrate (g)	5.0
$CaSO_4$ (g)	1.0
NH_4Cl (g)	1.0
Distilled water (mL)	400.0
Component 2	
K_2HPO_4 (g)	0.5
Distilled water (mL)	200
Component 3	
Sodium lactate (g)	3.5
Yeast extract (g)	1.0
Distilled water (mL)	400.0
Component 4	
$Fe(NH_4)_2(SO_4)_2$ (mL)	0.1

Test

Weight loss test: During the test, coupons were retrieved from anaerobic vials every 7 days (7, 14, 21 and 28). The coupons were cleaned with cleaning mineral solution (mixture of HCL and mineral solution) to remove all forms of dirt. The coupons were dried and their weights before and after immersion in the medium was recorded. Metal loss was determined by using the equation as follows (Yahaya *et al*., 2011; Norhazilan *et al*., 2012):

$$\text{Metal loss (W)} = (Wt\text{-}Wa)/A$$

where, Wt is initial weight of coupon (g), Wa is final weight of coupon (g) and A is area (cm^2).

Ultraviolet treatment: The above mentioned sample preparation was repeated with pH and temperature set at 9.5 and 37°C, respectively. The filled anaerobic vials were exposed to ultraviolet radiation at wavelength 254 nm (contact time is 60 min) and the sample was left to incubate at temperature 37°C for 22 days. Weight of steel coupons were recorded before and after the experiment. The turbidity of the SRB was taken on a daily basis until day 11 using spectrophotometer DR5000. Serial dilution method was used to ease the measurement of turbidity of SRB. In this experiment, turbidity has been chosen as the SRB growth indicator method.

RESULTS AND DISCUSSION

The coupons for this experiment were divided into 2 categories; the samples that had been exposed to SRB (named as SRB samples) and samples that were not exposed to SRB (named as control samples). After immersion periods, coupons were removed and cleaned with mineral solution and dried before weighing. Final weights of the steel coupons were taken to calculate the weight losses. The metal loss percent which defined by the percentage of weight loss of the steel were 0.37, 0.54,

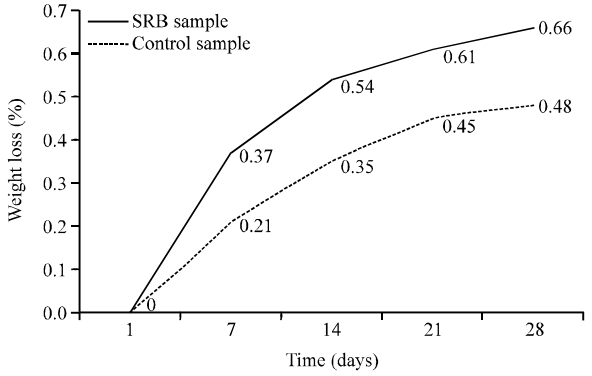

Fig. 1: Graph of weight loss (%) vs. days

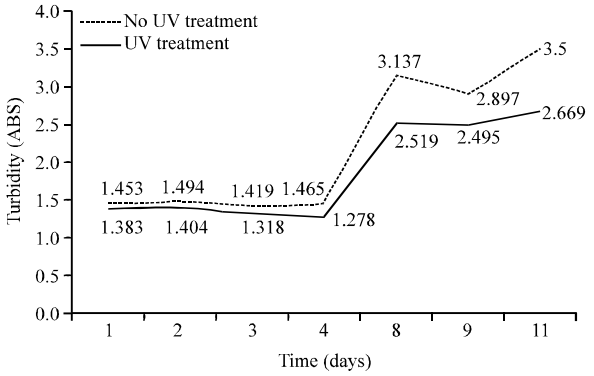

Fig. 2: Turbidity between samples treated and untreated using UV

Table 2: Turbidity on selected days and the difference of values between samples untreated and treated using UV

	Turbidity (ABS)		
Days	Untreated	Treated	Value difference (untreated-treated)
1	1.453	1.383	0.070
2	1.494	1.404	0.090
3	1.419	1.318	0.101
4	1.465	1.278	0.189
8	3.137	2.519	0.618
9	2.897	2.495	0.402
11	3.500	2.669	0.831

0.61 and 0.66% for SRB samples and 0.21, 0.35, 0.45 and 0.48% for control samples at 7, 14, 21 and 28 days, respectively.

Figure 1 displays the weight loss over time. The pH and temperature were fixed at 7 and 37°C, respectively. The pattern of the graph shows that metal loss volume increases over time for both samples. The rate of metal loss (slope gradient) seems to accelerate in the first 7 days and begins to decline thereafter due to depletion of resources for the bacteria to grow for SRB sample coupons. Metal loss also took place for the control samples but the rate was quite low as compared to the coupons that were immersed in SRB seed. The percentage differences of the two categories were 0.16, 0.19, 0.16 and 0.18% on day 7, 14, 21 and 28, respectively. After day 21, the metal loss of both samples had already started to increase slightly because of their source to induce corrosion has diminished. Theoretically, when bacteria is attached onto a steel surfaces, they start to form thin films known as biofilm (Kobrin, 1993) that consist of bacterial immobilization substratum, frequently implanted in an organic polymer matrix of microbial origin (Dexter and LaFontaine, 1998). Biofilm that started to form can influence corrosion in three general categories which are production of differential aeration or chemical concentration cells, production of organic and inorganic acids as metabolic byproducts and also production of sulfides under oxygen-free condition (Javaherdashti, 2004). When biofilm is developed, they start to change the electrochemical properties as well as physical properties of the metal (Javaherdashti, 2011). As for the SRB, the presence of ions such as sulfates and chlorides which were found in the medium supports the formation of biotically-generated sulfides in the corrosion products (Alabbas *et al*., 2012).Therefore, the longer the carbon steel is exposed to SRB, the more biofilm will be produced onto the coupons surface. This will cause an increasing amount in metal loss and cause corrosion such as pitting dependent on time.

In order to test the disinfection efficiency of UV treatment upon SRB growth, turbidity of the SRB samples were recorded on selected days. Higher turbidity shows the higher population of SRB presence. The characteristic of unpleasant smells from hydrogen sulfide and black coloured solution were the evidence of SRB growth and its metabolism in the medium (Postgate, 1984). Table 2 lists the data of turbidity on selected days and the difference of value between untreated and treated samples using UV. Based on the data, it clearly shows that turbidity value of all the samples decreased after the UV treatment.

Figure 2 show the clear view of the UV wave treatment effectiveness. The disinfection of SRB growth using UV waves proved to be effective due to the decrease in turbidity value after the treatment. Figure 3 illustrates the treatment efficiency in percentage over time. According to Wang *et al*. (2005), UV wavelength that is considered as the most effective germicidal with the germicidal activity peaks at 254 nm which compare well with the UV wavelength used in this study. From the graph pattern, it indicates that the treatment efficiency increases over time. It can be stated that the longer the samples exposed to the UV wave, the higher the number of SRBs that could be exterminated. The most effective treatment time is day 11 because it has the highest difference of turbidity value.

The statistical analysis of the data is shown in Table 3 and 4. The numerical correlation value for paired sample correlation between the two paired samples for untreated and treated of metal loss and also untreated and treated of turbidity using ultraviolet is 0.989 and 0.992, respectively. From this value, indicates the presence of strong relationship between the pairs. Table 4 stated the paired samples test of T-Test. The Sig. (2-Tailed) value in the table is 0.017, Pair 1 and 0.027, Pair 2. This value is less than 0.05 so there is a statistically significant difference between the mean of metal loss and turbidity before and after the samples treated with ultraviolet.

Table 3: Paired samples correlations

Pair	N	Correlation
1 (untreated and treated)	5	0.989
2 (untreated UV and treated UV)	7	0.992

Figure 4 illustrated the Field Emission Scanning Electron Microscopy (FESEM) image of corrosion product

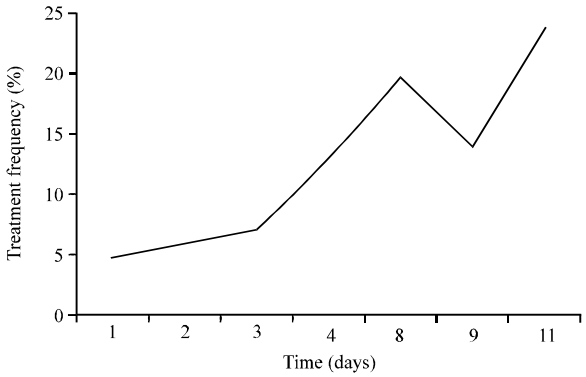

Fig. 3: UV treatment efficiency vs. time

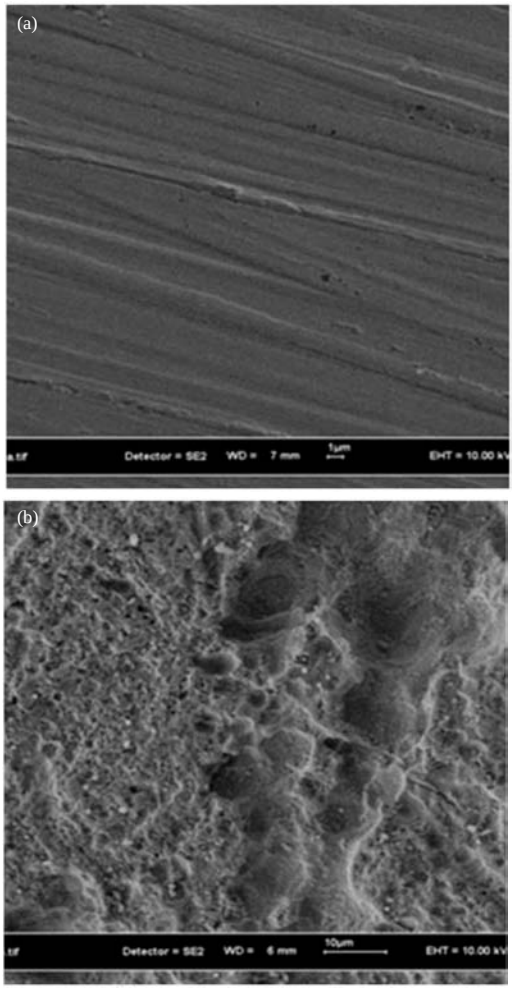

Fig. 4(a-b): FESEM image of corrosion product on the surface of coupon, (a) Before exposure to SRB and (b) After exposure to SRB (corrosion product)

Table 4: Paired samples test

	Paired differences							
				95% confidence interval of the difference				
Pair	Mean	SD	SEM	Lower	Upper	t	df	Sig. (2-tailed)
1 (untreated-treated)	0.138000	0.78230	0.034990	0.040860	0.235140	3.944	4	0.017
2 (untreated UV-treated UV)	0.328429	0.299222	0.113095	0.051695	0.605162	2.904	6	0.027

on the surface of coupon before and after being exposed to SRB. Before exposure to SRB, the surface of the carbon steel had been cleaned to a mirror surface by ethanol degreasing and the above image in Fig. 4 showed the FESEM image of carbon steel surface microstructure without any substances. However, after 28 days of immersion in the medium that contains SRB, the biofilm and corrosion product has been graphically traced on the coupon surfaces. The substrate of the steel was scarcely visible since it was covered with a porous black layer. The black layer was the evidence of SRB metabolism activities on the metal surface. The jelly-glue substance, a by-product of corrosion was also traced by FESEM. In order for MIC to happen, the corrosion process starts with the biofilm formation on the metal surface. SRB cells will attach to the coupon surface, grow, reproduce and produce an Extracellular Polymer Substance (EPS). According to previous research reports, normally the EPS and corrosion products occupied 75-95% of biofilm volume while 5-25% is occupied by the cells (Alabbas *et al*., 2012).

CONCLUSION

Based on these results, it is reasonable to assume that SRB can cause severe corrosion damage on the Carbon Steel Grade API 5L-X70. The metal loss experiment has indicated that metal loss increases linearly over time of exposure to the SRB. Turbidity measurement has proved that UV wave could mitigate the growth of SRB. The implementation of UV wave during the treatment process can be one of the potential Non-physical Inhibitory (NPI) method that is cordial to the environmental, cost effective and helps in reducing chemical substances usage to disinfect microorganism specifically SRB.

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