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Combination effects of ultrasound wave and biocide treatment on the growth of sulfate reducing bacteria (SRB)

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ABSTRACT

Microbiologically influenced corrosion (MIC) is caused by the presence of sulfate-reducing bacteria (SRB) and is of great concern to the heavy metal industries. Inhibitors and biocides are commonly used to control the problem. Nevertheless, the solutions are too expensive and may create environmental problems by being corrosive to metals. Ultrasound wave exposure is one of the potential alternatives to biocides for the mitigation of MIC risk in pipeline system. In this work, a combination of ultrasound wave and green biocides of glutaraldehyde and ethanol has been proposed to exterminate SRB in a medium. An amount of 100 ml of *Desulfovibrio vulgaris* (ATCC7757) broth number 1249 was grown in 125 ml anaerobic vials at 37°C for one day followed by exposure to various mitigation methods. Results from the study show that a combination of ultrasound and biocide can effectively reduce the dosage of biocide during corrosion treatment. The effectiveness of mitigation based on ultrasound-biocide combination is better than the treatment based solely on biocide whereby the extermination of SRB was found 10 times more effective according to the reduction of cell numbers of planktonic's SRB. Ultrasound technique can provide a feasible alternative as an effective assist to chemical inhibitors and biocides for controlling MIC in a more eco-friendly manner.

Keywords: Sulfate-reducing bacteria; Ultrasound wave; Green biocide mitigation

1. Introduction

Corrosion is defined as the destruction or deterioration of material due to the reaction with its surrounding environment. Corrosion problems are a major issue in the operation and maintenance of oil and gas industry pipelines [1] and have inflicted huge cost of repair and maintenance to the industry. It was reported that the annual cost of all forms of corrosion to oil and gas industry was estimated as $13.4 billion in 2001 [2]. The corrosion of pipelines, tanks, storage units, and associated equipment increases the risk of the release of hazardous materials to the environment, with concomitant pollution issues [3].

Microbiologically influenced corrosion (MIC) is defined as a degradation of material under the influence of environmental factors complicated by the metabolic activities of micro-organisms. It is a widespread problem in oil and gas, paper, and nuclear industries. Considerable efforts were implemented to control it, either through the use of corrosion inhibitors or biocides [4–6].

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Sulfate-reducing bacteria (SRB), known as *Desulfovibrio vulgaris*, is one of the most destructive micro-organism that can cause corrosion because of its ability to reduce sulfate or sulfite ions present in the media to sulfide ions [7]. Sulfide produced by SRB is the most reduced form of sulfur and is highly soluble and reactive [8]. These bacteria are nonpathogenic and anaerobic in nature. They produce enzymes which have the power to accelerate the reduction of sulfate compounds to the corrosive hydrogen sulfide. In other words, SRB acts as a catalyst in reduction reaction. There are two types of SRB, namely planktonic, which is free floating in the system, and sessile bacteria, which are adherent and attached to the surface.

Biocide treatments, such as glutaraldehyde (GTD), cocodiamines, and tetrakis hydroxymethyl phosphonium sulfate (THPS) are widely used to mitigate MIC in steel pipes and in closed systems [9]. Cathodic protection is also used successfully to prevent MIC when used with coating [10]. However, the use of biocides and cathodic protection techniques is very expensive for the industry [11,12] and some studies suggest that water-soluble inhibitors should be avoided in petroleum product transporting pipelines [13]. Biocides can also cause environmental pollution in terms of chemical wastage and they may be corrosive to metals [14]. To avoid unintended chemical corrosion caused by biocides,

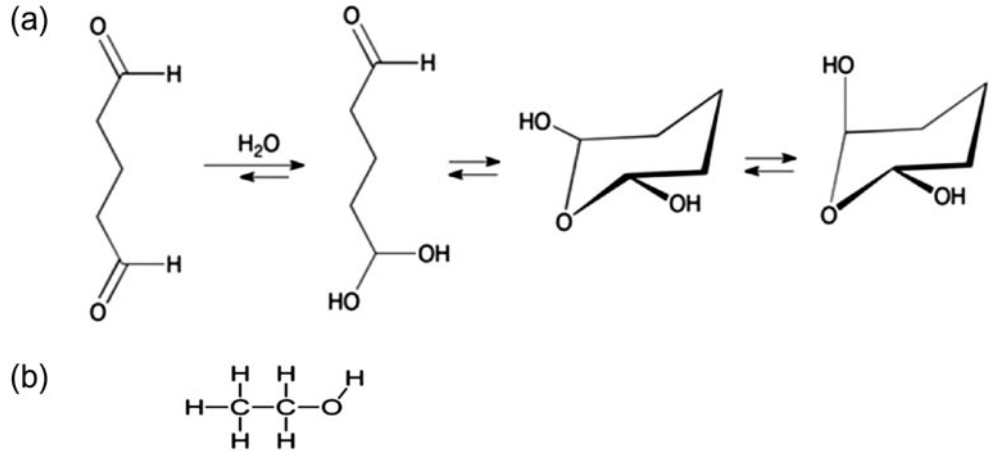


Fig. 1. Structure of biocide: (a) GTD [18] and (b) Ethanol [19].

Table 1
Test matrices for GTD and ethanol and combination with mechanical treatment (ultrasound) of planktonic SRB

Test	Vials numbers	SRB	500 ppm Glutaraldehyde	1000 ppm Glutaraldehyde	5% Ethanol	10% Ethanol	Ultrasound (min)	
							15	30
1	5	2 ml						
2	5	2 ml	1 ml of 5%		5.3 ml of 95%			
3	5	2 ml	1 ml of 5%		5.3 ml of 95%		Yes	
4	5	2 ml	1 ml of 5%		5.3 ml of 95%			Yes
5	5	2 ml	1 ml of 5%			10.6 ml of 95%		
6	5	2 ml	1 ml of 5%			10.6 ml of 95%	Yes	
7	5	2 ml	1 ml of 5%			10.6 ml of 95%		Yes
8	5	2 ml		2 ml of 5%	5.3 ml of 95%			
9	5	2 ml		2 ml of 5%	5.3 ml of 95%		Yes	
10	5	2 ml		2 ml of 5%	5.3 ml of 95%			Yes
11	5	2 ml		2 ml of 5%		10.6 ml of 95%		
12	5	2 ml		2 ml of 5%		10.6 ml of 95%	Yes	
13	5	2 ml		2 ml of 5%		10.6 ml of 95%		Yes

nonoxidizing biocides are favoured in the heavy metal industries [15]. GTD is the most popular non-oxidizing biocide. They are classified under broad-spectrum and biodegradable biocide. As explained by Reza [11], a broad-spectrum biocide must be able to kill as many diverse types of micro-organisms and as many of the same type of micro-organism as possible.

More environmental methods are under consideration as an alternative to biocides, one such approach is by using ultrasound [5]. Ultrasound is a cyclic sound pressure with a frequency greater than the upper limit of human hearing. Although this limit varies from person to person, the lower limit is approximately 20 kHz and this serves as a useful lower limit to describe ultrasound [13]. Reza [11] also explained that the production of ultrasound is used in many different fields, typically to penetrate a medium and measure the reflection signature or supply focused energy. Although ultrasound is primarily a mechanical method to mitigate bacteria, thermal and chemical effects also contribute to microbial sterilization.

The present study was conducted by evaluating the impact of combination of biocide and ultrasound treatment to remove corrosion causing micro-organism. The objective of this work is to determine whether an ultrasonic system can provide a feasible alternative of an effective adjunct to the chemical inhibitors and biocides for controlling MIC. As most of the previous studies were mainly focusing on mitigation by using either biocides [2,4] or ultrasound [3,4], this paper is focusing on the combination of both methods in the treatment of SRB. The main goal is to identify the efficiency of ultrasound in reducing the

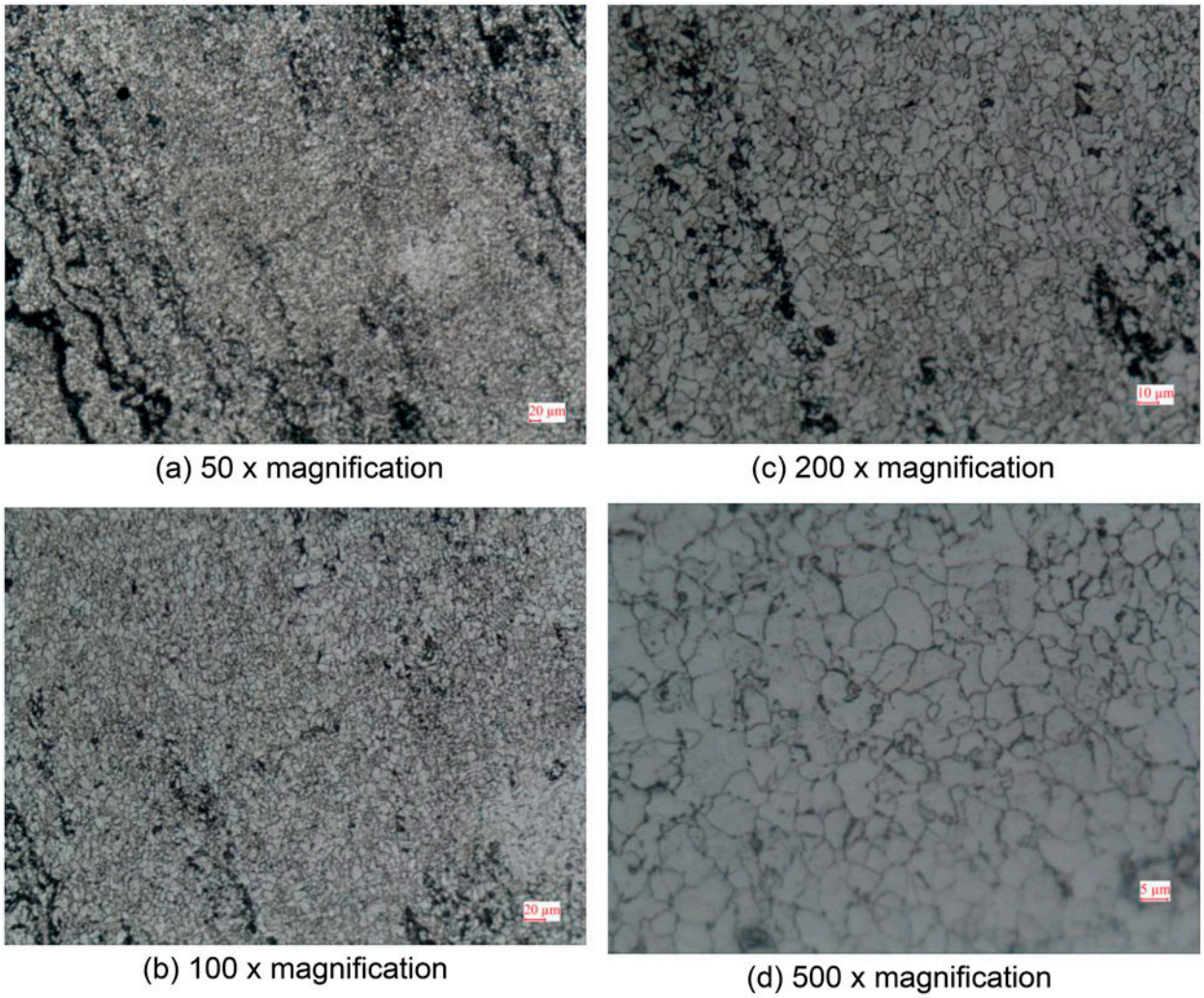


Fig. 2. Optical micrograph of carbon steel API 5L grade x70 coupon sample (a) 50x magnification, (b) 100x magnification, (c) 200x magnification, and (d) 500x magnification.

amount of biocide to mitigate the MIC without jeopardizing the proven effectiveness of biocide treatments.

2. Experimental procedure

2.1. Coupon preparation

Coupons were prepared using carbon steel pipe grade API 5L-X70 (specimens were machined from the actual segment of pipe API 5L X-70 obtained from local gas operator). The coupons were refined with 100 grit Si–C paper, cleaned, and dried with ethanol to remove all forms of dirt, grease, and small Si–C particles on the coupons surface. The cleaned and dried coupons were then coated with prime coat leaving only the top surface exposed. The coupons then were dried overnight in an oven at 37˚C. The exposed area of the coupons was polished again with series of Si–C papers grade (320, 600, and 800), followed by ethanol degreasing. Image analyser was used to observe the morphology of carbon steel coupon size 10 m × 20 m. Biofilm on the coupon surface was observed by field-emission scanning electron microscopy (FESEM) (Gemini SUPRA^{TM} FESEM 35 VP).

2.2. Culture and medium

The strain *D. vulgaris* ATCC 7757 is a marine strain of SRB and was grown in a modified ATCC broth number 1249 (Modified Barr's Medium). The anaerobic condition was created by purging filtered nitrogen [16,17]. The medium for SRB growth composes of the following chemicals (per litre of distilled water): sodium lactate 4.5 ml, yeast extract 1.0 g, potassium di-phosphate (K_2HPO_4) 0.5 g, magnesium sulfate ($MgSO_4$) 4.096 g, sodium citrate ($2H_2O$) 5.7 g, calcium sulfate monohydrate ($CaSO_4.2H_2O$) 1.27 g, and ammonium chloride (NH_4Cl), 1.0 g. The pH was adjusted to 7.0 with 1 M sodium hydroxide (NaOH).

The prepared medium was then sterilized for 20 min at a pressure of 1.2×10^4 Mpa. After the medium cooled down, 2 ml ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)_2$ was sterilized with 0.2 μm filter and added separately. The SRB was grown in this 100 ml of medium contained in 125 ml vials at 37˚C under anaerobic conditions. Two chemical-based biocides, GTD and ethanol, were used in the experiment. GTD, an organic compound ($CH_2(CH_2CHO)_2$) and ethanol, a straight chain alcohol made from fruit or sugar containing materials, such as molasses with molecular formula of C_2H_5OH, are shown in Fig. 1(a,b).

Nitrogen sparging was used to remove the oxygen present in the medium. Different concentrations of GTD and ethanol were added to vials before inoculation (Table 1). A 100 ml of two-day old SRB stock culture was used to inoculate each vial. Statistically, five vials were used for every single difference in treatment concentration in order to obtain the accurate

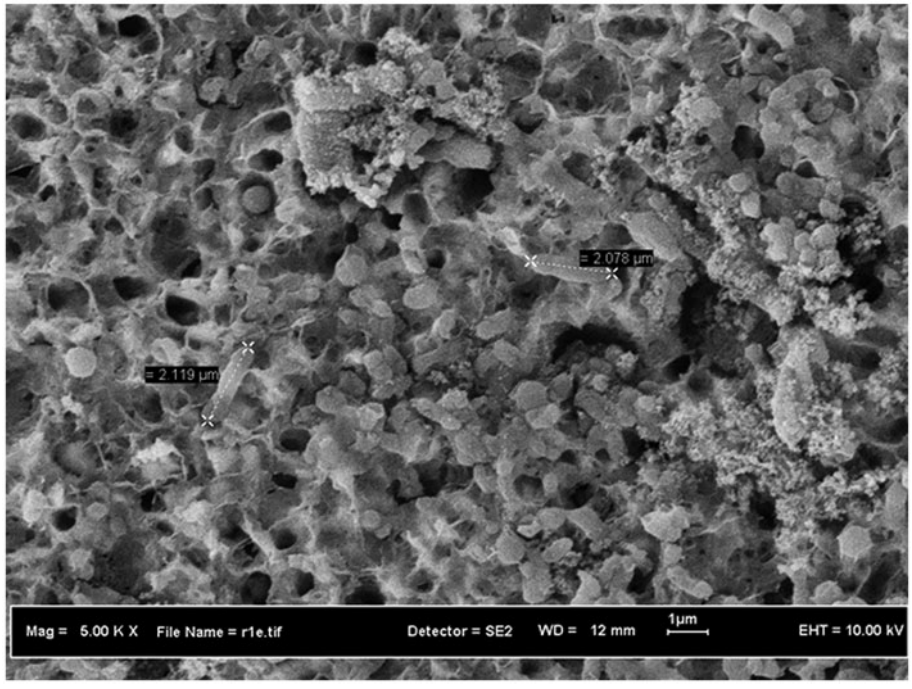
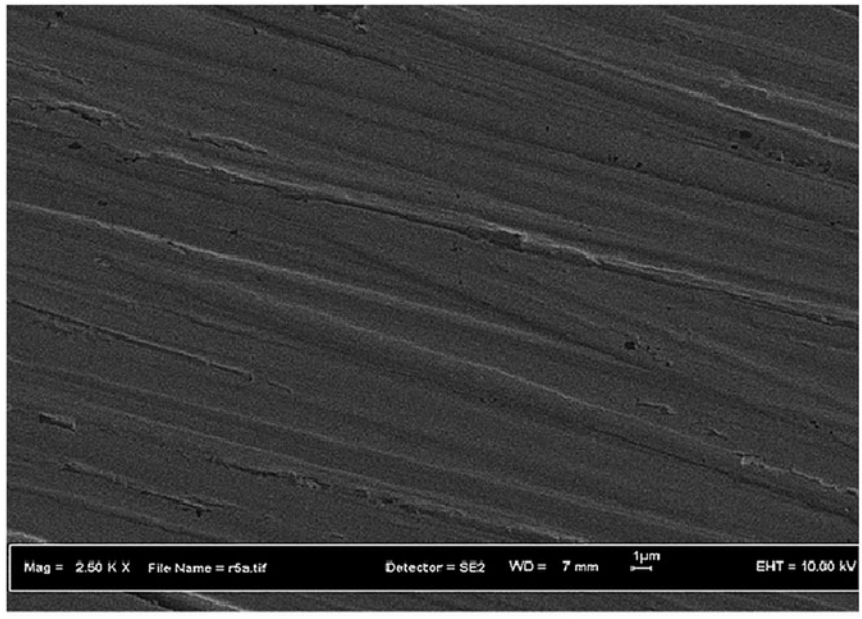
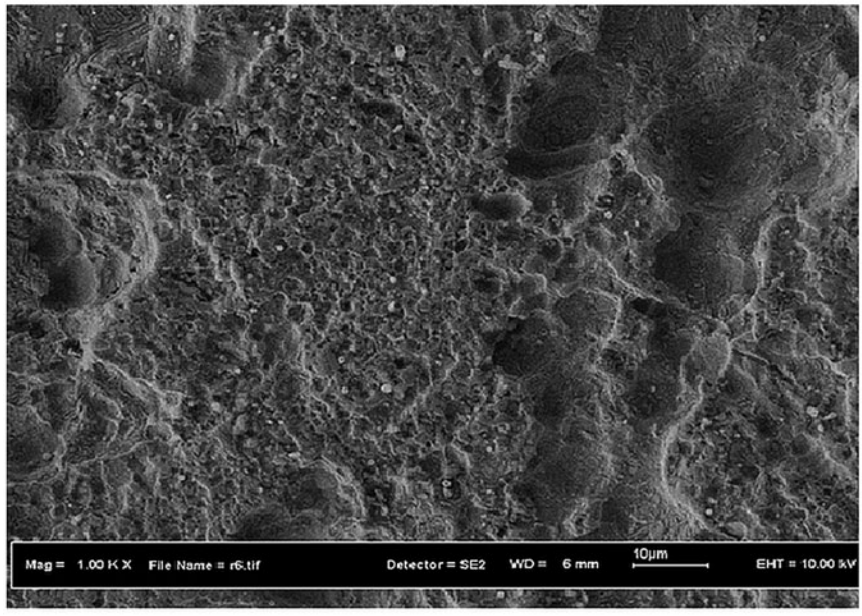


Fig. 3. FESEM image of SRB biofilm.



(a) Before exposure to SRB



(b) After exposure to SRB

Fig. 4. FESEM image of corrosion product on the surface of coupon (a) before exposure to SRB and (b) after exposure to SRB (corrosion product).

data from average number of cells. The SRB cell concentration was measured by diluting the sample. It was then followed by a process of calculation of the planktonic of SRB using hemocytometer method and microscope. The concentration after inoculation was estimated to be 2.0×10^6 cells/ml.

An anaerobic laminar flow chamber with a nitrogen environment was used to provide an anaerobic environment for inoculation. A 100 ml of medium was distributed into each vial with an appropriate amount of a biocide, followed by exposure to low-frequency ultrasound wave for 15 and 30 min

separately. The initial SRB cell concentrations were estimated and the vials were sealed and then placed in an incubator at 37°C. SRB growth was measured by counting the numbers of cells using a hemocytometer under optical microscope at 400x magnification.

3. Results and discussion

3.1. Surface analysis

Fig. 2 illustrates the microstructures of the carbon steel API 5L grade 70 (X70) under different levels of magnification using image analyzer. It shows that X70 has refined grain structure indicative of its greater strength as compared to lower grade of carbon steel. The smaller grain size steel has a higher susceptibility to MIC [20]. Fig. 3 depicts the *D. vulgaris* strain ATCC 7757 biofilm on the coupon steel being examined by FESEM. Biofilm can develop when the planktonic bacteria is attached to the coupon surface [21]. The formation of H_2S in the presence of SRB can form a temporary protective layer for a short period of time that may cause pitting corrosion under anaerobic conditions [20,22–24].

Fig. 4 shows the coupon surface before and after exposure to SRB colony. After exposure to the SRB for certain period of time, the surface of steel coupon was fully covered by the produced corrosion product originated from SRB metabolic activities.

3.2. Comparison of treatment concentration

GTD is a widely used biocide in oil fields, as are THPS, quaternary ammonium compounds, and bromo-nitropropanediol. Due to its broad-spectrum and biodegradability, GTD was selected for this study. GTD can act as a suppressor for SRB cell growth

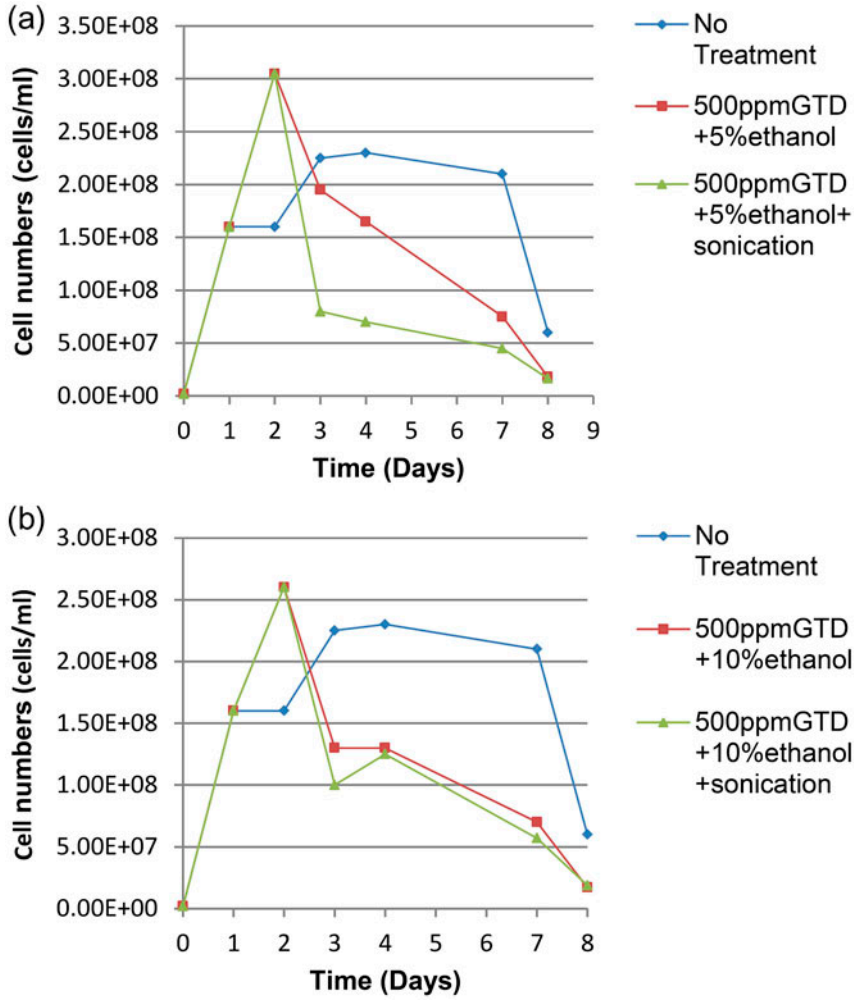


Fig. 5. Effects of 500 ppm glutaradehyde and ethanol on the number of cells of SRB in sonicated and unsonicated medium with 15 min exposure: (a) 5% ethanol and (b) 10% ethanol.

because of its interaction with the SRB culture medium [25]. The second type of biocide, ethanol, was selected due to the miscibility of ethanol with water. This contrasts with that of longer chain alcohols (five or more carbon atoms).

The differences of mitigation efficiency using biocide treatments, sonication and biocide treatments, and with and without treatment results are shown in Figs. 5–8 with different concentrations of GTD and ethanol.

Referring to the results of SRB growth without treatment, the growth process of SRB was characterized by a rapid growth and decline period. SRB growth started to increase approximately from day-1 to day-3. During this stage, the concentration of hydrogen sulfide (H_2S) increased proportionally according to the increasing number of cells [25,26]. As nutrients became insufficient, the cell density naturally declined [27]. After day-3, the cell numbers of SRB started to decrease due to the depletion of hydrogen sulfide (H_2S), inhibiting the growth of SRB. The bacteria count dropped drastically after day-7 and marked the end of the experiment. The characteristics of unpleasant smells from hydrogen sulfide and black-colored solution were the evidence of SRB growth and its metabolism in the medium [26].

Fig. 5 displays the result of bacterial growth in sonicated (15 min of exposure) and unsonicated medium with different concentrations of ethanol. The results show that the number of bacteria increased rapidly over samples without treatment on day-2 and the bacterial cell number slowly decreased thereafter. As opposed to results from untreated medium, the bacteria count drastically dropped from day-2 and almost reached full extermination extinction after day-7. The extermination of the bacteria is due to

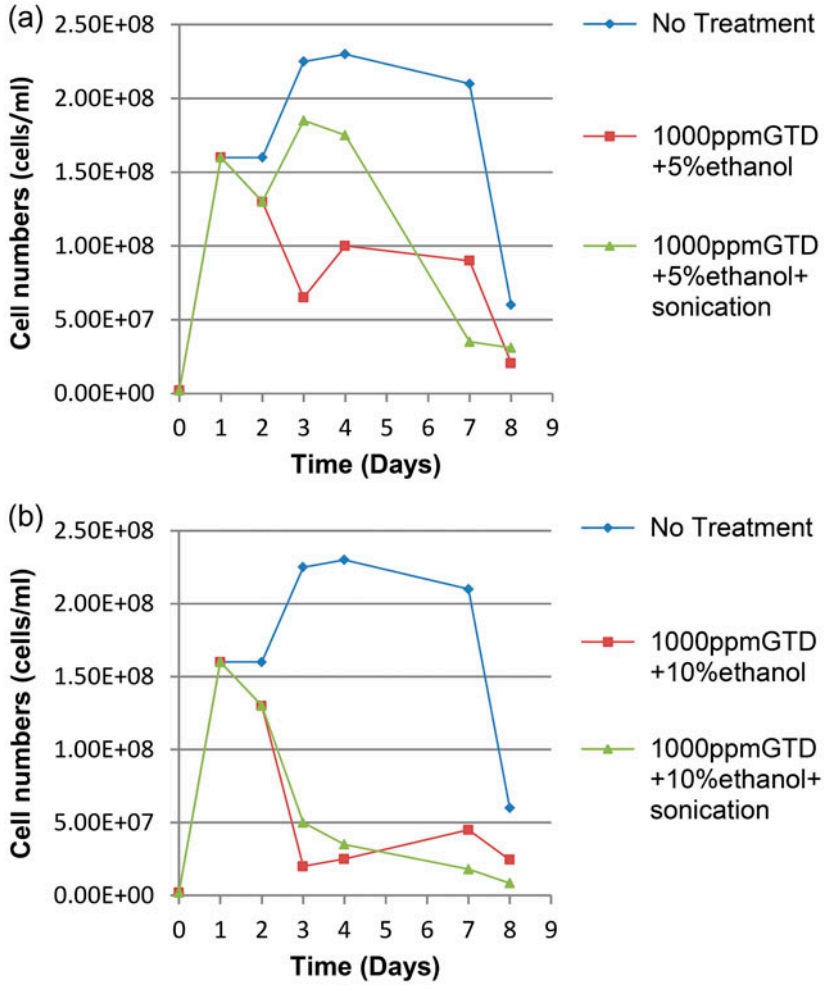


Fig. 6. Effects of 1,000 ppm GTD and ethanol on the number of cells of SRB in sonicated and unsonicated medium with 15 min exposure (a) 5% ethanol and (b) 10% ethanol.

sonication and biocide treatment assisted by the depletion of nutrient on day-7. This reflects the efficiency of sonication-biocide combined treatment to reduce bacterial cell density without relying on nutrient depletion. At 5% ethanol dosage, sonication and biocide treatment was found to be more effective in the extermination of bacteria from day-2 to day-7. The comparable performance between sonication-biocide combined treatment and biocide only could be achieved by increasing the ethanol dosage to 10%.

The next step was to examine the effects of broad-spectrum biocide (GTD) on bacterial extermination by doubling the GTD concentration to 1,000 ppm, whilst maintaining the ethanol dosage and 15 min of exposure to ultrasound. Based on comparison between results from Figs. 5(b) and 6(b), it is noticeable that the doubled amount of concentration of GTD at 10% level of ethanol has increased the rate of removal of bacteria almost twice. There is not much difference in terms of performance between sonicated and unsonicated medium at 10% dosage of ethanol, even though sonication did show lesser effect according to the lower bacteria count obtained on day-7. The whole setup of the experiment was repeated with a higher exposure of medium to ultrasound. The time was raised from 15 to 30 min. Overall results from Figs. 7 and 8 indicate that a higher period of exposure did slightly improve the performance of bacteria removal.

Bacteria disinfection through mitigation process using combination of mechanical treatment (ultrasound) and biocides was performed to inhibit the growth of SRB in the medium. Sonicated medium was found to have the least number of bacterial counts on day-7 for all setup of experiments. This reflects the efficiency of mechanical treatment together with biocide in suppressing the growth of SRB. The

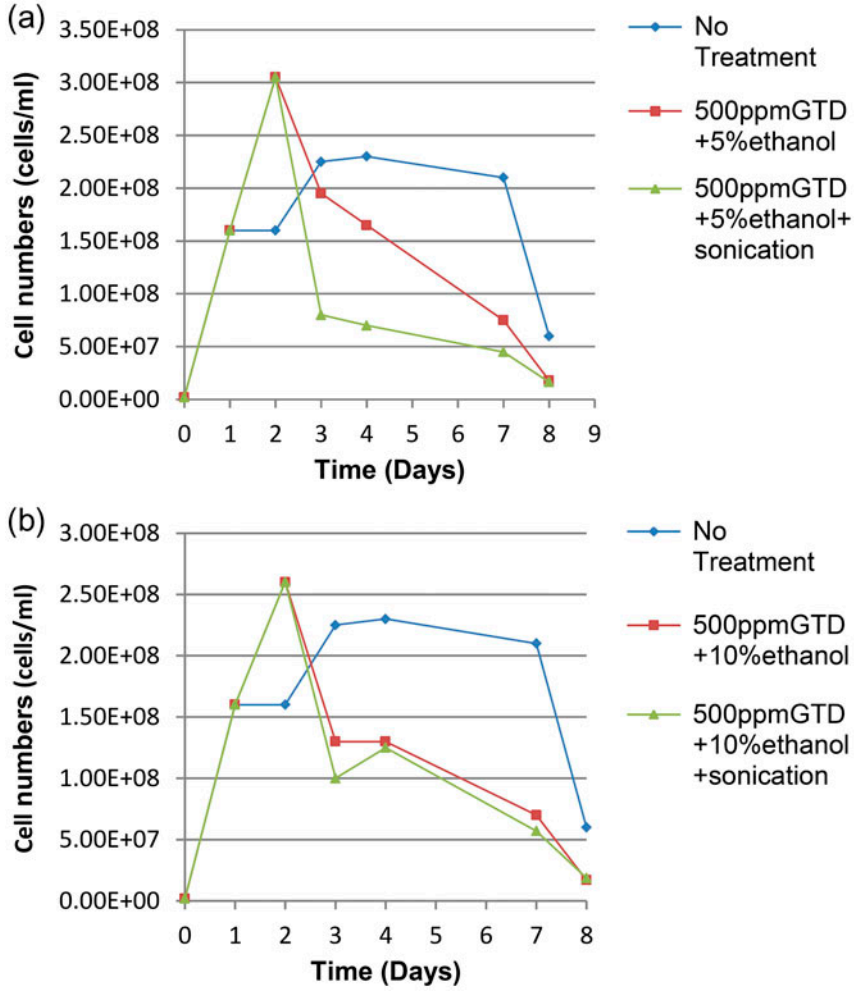


Fig. 7. Effects of 500 ppm glutaradehyde and ethanol on the number of cells of SRB in sonicated and unsonicated medium with 30 min exposure (a) 5% ethanol and (b) 10% ethanol.

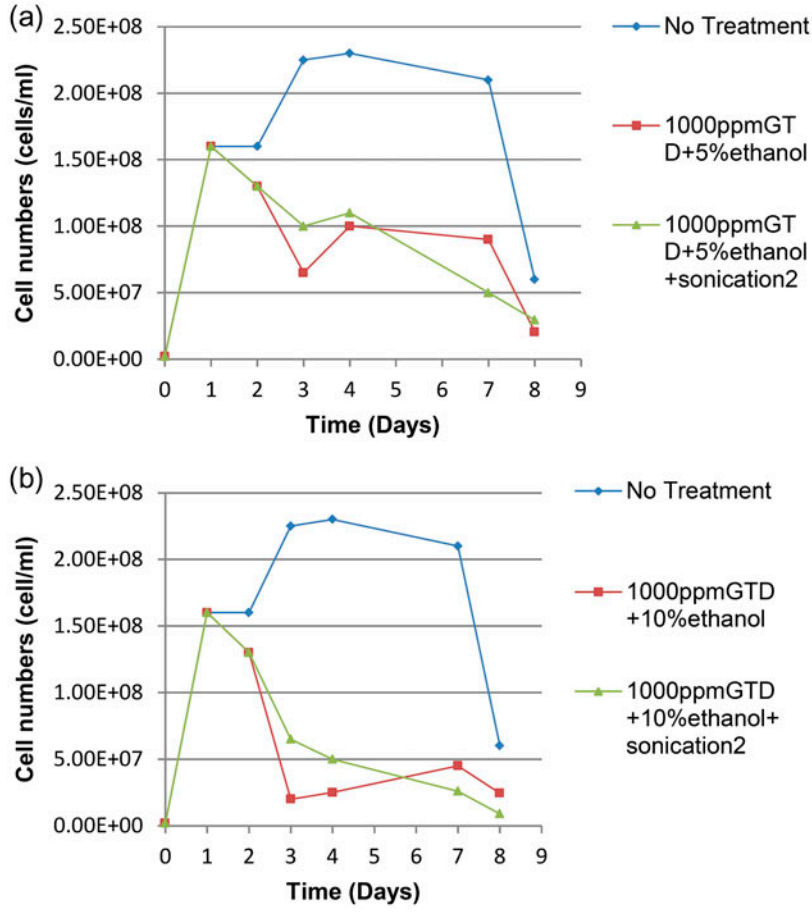


Fig. 8. Effects of 1,000 ppm gluthradehyde and ethanol on the number of cells of SRB in sonicated and unsonicated medium with 30 min exposure (a) 5% ethanol and (b) 10% ethanol.

increment of biocide dosage has overpowered the sonication, whereby the performances of unsonicated mediums are almost comparable with sonicated medium. Sonication process seems to have better influence on bacteria disinfection at low dosage of biocide when GTD and ethanol were set at 500 ppm and 5%, respectively. Time of exposure to ultrasound played less important role in the efficiency of this mitigation treatment, since there is no distinct difference of disinfection efficiency between 15 and 30 min of exposure.

The findings prove that ultrasound can be used with lower dosage of biocide to disinfect SRB in the medium. The combination of biocide and ultrasound treatment can reduce bacterial numbers to very low levels within few minutes of exposure. The reduction of biocide amount in the treatment can minimize the chemical wastage as well as reduce the corrosiveness of the treatment toward steel.

4. Conclusions

Environmental concerns have prompted researchers to find alternative ways to mitigate MIC, other than fully depending on chemical and abrasive biocides. This research has provided valuable information regarding the performance of ultrasound with low dosage of biocide in SRB mitigation treatment. The use of a higher range of ultrasound or more exposure time are two more variables that needs to be considered in the future to achieve mitigation of SRB that are high enough to have a marked effect on corrosion. The corrosion of pipeline steel in a flowing medium with SRB present may be suppressed by combination ultrasound and biocide. However, the overall efficiency of the proposed hybrid treatment still depends on the concentration of bacteria surviving and becoming recontaminated during treatment.

Acknowledgments

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References

[1] B.J. Little, B.P. Wagner, F. Mansfeld, An overview of microbiologically influenced corrosion, Electrochim. Acta 37(12) (1992) 2185–2194.
[2] G.H. Koch, M.P.H. Brongers, N.G. Thompson, Y.P. Virmani, J.H. Payer, Corrosion Cost and Preventive Strategies in the United States, FHWA-RD-01-156, Washington, DC, Federal highway Administration, 2001.
[3] F. Aktas Deniz, S. Lee Jason, J. Little Brenda, Richard I. Ray, Irene A. Davidova, Christoper N. Lyles, Joseph M. Suflita, Anaerobic metabolism of biodiesel and its impact on metal corrosion, Energy Fuels 24 (2010) 2924–2928.
[4] P.A. Vivekanand, M.l. Wang, Ultrasound-assisted N-alkylation of Phthalimide under phase-transfer catalysis conditions and its kinetics, Chem. Eng. Commun. 199(12) (2012) 1652–1667.
[5] B.G. Pound, Y. Gorfu, P. Schattner, K.E. Mortelmans, Ultrasonic mitigation of microbiologically influenced corrosion, Corrosion 61(5) (2005) 452–463.
[6] J. Wen, D. Xu, T. Gu, I. Raad, A green biocide cocktail consisting of a biocide, EDDS and methanol for the mitigation of planktonic and sessile sulphate-reducing bacteria, World J. Microbiol. Biotechnol. 28(2) (2011) 431–435.
[7] J. wen, D. Xu, T. Gu, I. Raad, Chelators enhanced biocide inhibition of planktonic sulphate-reducing bacteria growth, World J. Microbiol. Biotechnol. 26(6) (2009) 1053–1057.
[8] H.L. Johnston, G. Voordouw, Sulfate-reducing bacteria lower sulfur-mediated pitting corrosion under conditions of oxygen ingress, Environ. Sci. Technol. 46(16) (2012) 9183–9190.
[9] Y.Z. Xiang, H. Modi, J. John Kilbane, Efficacy and risks of nitrate application for the mitigation of SRB-induced corrosion, Corros. Nace. Expo. (2006). 61st Annual Conference and Exposition. Paper No. 06524.
[10] M.G. Fontana, Corrosion Engineering, 3rd ed., McGraw-Hill, New York, NY, 1986.
[11] J. Reza, Microbiologically Influenced Corrosion an Engineering Insight, Springer-Verlag, London, 2008.
[12] E.S. Pankhurst, Significant of sulphate-reducing bacteria to the gas industry: A review, J. Appl. Bacteriol. 31 (1968) 179–193.
[13] M. Sundaram, K.B. Dinesh, R. Shanmugavel, A. Balakrishnan, P. Seeni, C. Maruthai, S. Palani, P. Narayanan, Microbial corrosion in petroleum product transporting pipelines, Ind. Eng. Chem. Res. 50(13) (2011) 8006–8015.
[14] R. Cord-Ruwisch, W. Kleinitz, F. Widdel, Sulfate-reducing bacteria and their activities in oil production, J. Petrol Technol. 39 (1987) 97–105.
[15] M.J. Franklin, D.E. Nivens, A.A. Vass, M.W. Mittelman, R.F. Jack, N.J.E. Dowling, D.C. White, Effect of chlorine and chlorine/bromine biocide treatment on the number and activity of biofilm bacteria and on carbon steel corrosion, Corrosion 47 (1991) 128–134.
[16] I. Laopaiboon, N. Phukoetphim, P. Laipaiboon, Effect of glutaradehyde biocide on laboratory scale rotating biological contactors and biocide efficacy, Electron. J. Biotechnol. 9 (2006) 358–369.
[17] An. Hu, Investigation of Sulfate-reducing Bacteria Growth Behavior for the Mitigation of Microbiologically Influnced Corrosion (MIC), Thesis Master of science, Ohio University, 2004.
[18] M.J. Karnvsky, A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy, J. Cell Biol. 27 (1965) 137A–138A.
[19] B. Boggan, Effects of Ethyl Alcohol on Organ Function, (2007). Available from: Chemases.com.
[20] M. Alabbas Faisal, J.R. Spear, D.L. Olson, A.B Gavanluei, A. Kakpovbia, B. Mishra, Effects of sulfate reducing bacteria on the corrosion of X-65 pipeline carbon steel, Corrosion (2012), Paper No. C2012-0001140.
[21] J. Liu, X. Liang, S. Li, Effect of sulphate-reducing bacteria in the electrochemical impedance spectroscopy characteristic of 1Cr18Ni9Ti, J. Univ. Sci. Technol. Beijing 14(5) (2007) 425–430.
[22] H.H. Almahamedh, C. Williamson, J.R. Spear, B. Mishra, D.L. Olson, Identification of micro-organism and their effects on corrosion of carbon steels pipelines, Corrosion (2011), NACE International Conference and Expo. Paper No. 11231.
[23] M. Murto, L. Bjornsson, B. Mattason, Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure, J. Environ. Manage. 70 (2011) 101–107.
[24] N. Yahaya, K.S. Lim, N.M. Noor, S.R. Othman, A. Abdullah, Effect of clay and moisture content on soil corrosion dynamic, Malaysia J. Civ. Eng. 23(1) (2011) 24–32.
[25] B.J. Little, P. Wagner, K. Hart, R. Ray, D. Lavoie, The role of metal-reducing bacteria in microbiology influenced corrosion, Corrosion (1997), Paper No. 215.
[26] B.J. Little, J.S. Lee, Microbiologically Influenced Corrosion, John Wiley & Sons, Hoboken, NJ, 2007.
[27] M. Madigan, Brock Biology of Microorganism, 12th ed., Pearson Benjamin Cummings, San Francisco, 2009.