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# APPLICATION OF ULTRAVIOLET RADIATION TO CONTROL MICROBIOLOGICALLY INFLUENCED CORROSION: A CASE STUDY ON SOIL SAMPLE FROM MANGROVE

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**Abstract**

Microbiologically influenced corrosion (MIC) is significant to the presence of microorganisms such as sulfate reducing bacteria (SRB) in the deterioration of metallic and non-metallic materials. Bacterial chemical biocides are commonly used to disinfect microorganism effectively. Yet, the practice has some negative impact on the environment since the chemical content may cause pollution. A laboratory experimental investigation was conducted to explore the performance of Ultraviolet (UV) radiation in exterminating SRB as an option to replace biocides usage. The morphologies of the isolated Sg. Ular SRB used in this study were related to *Desulfovibrio* species. An experimental work was conducted in determining the optimum pH and temperature for the SRB to grow before disinfection purposes. The experimental result showed optimum growth for respective SRB were at pH of 8.5 with temperature recorded at 37˚C. UV radiation with wavelength of 254 nm was utilised to disinfect the microorganism. SRB samples were exposed to UV radiation for one hour and left incubated for 21 days. It was found that the percentage of metal loss in a sample exposed to UV radiation was lower compared with untreated sample. Results from the study revealed that UV has a potential as a viable option for SRB disinfection purposes and may be further developed to reduce the consumption of chemical biocides in pipeline maintenance scheme.

Keywords: Sulphate reducing bacteria (SRB); Corrosion; Microbiologically influenced corrosion (MIC); Ultraviolet (UV)

**Nomenclatures**

| | |
|---|---|
| *Wa* | Final weight of coupon, g |
| $W_o$ | Initial weight of coupon, g |

**Abbreviations**

| | |
|---|---|
| API | American Petroleum Institute |
| DNA | Deoxyribonucleic acid |
| MIC | Microbiologically influenced corrosion |
| PCR | Polymerase chain reaction |
| SiC | Silicon carbide |
| SRB | Sulfate reducing bacteria |
| UV | Ultraviolet |

## 1. Introduction

In the oil and gas industry, transmission pipelines have an excellent safety record to facilitate bulk shipments of crude oil and natural gas to consumers worldwide. However, it has properties like other engineering structure which can and will fail. These failures can be caused by mal-operation, outside force, corrosion, pilferage and other factors. Consequently, a pipeline operator should be able to inspect and maintain the pipeline thoroughly and regularly to ensure that it is not deteriorating or damaged within its intended designed life [1]. Corrosion is a type of deterioration or damage due to chemical reactions of metals to the environment which is unavoidable although the effect can be minimised through a proper maintenance strategy [2]. Corrosion is an economic and engineering problem. It is also among the primary factors affecting the longevity and reliability of pipelines that transport crucial energy sources throughout the nation.

Deterioration of metal by corrosion process directly or indirectly involving microorganisms has gained serious attention from pipeline operators recently. A few terms have been used by the engineers and the scientific community to highlight this type of corrosion, such as biocorrosion, biodeterioration and microbiologically influenced corrosion (MIC) [3, 4]. As indicated by United States Department of Transportation Office of Pipeline Safety, internal corrosion caused 15% of all reportable incidents affecting gas transmission pipeline over the past several years, leading to an average cost of 3 million dollars annually in property damage and several fatalities [5]. Thus, the prevention and protection of MIC has become an important problem that is required to be solved [6]. The most highlighted and catastrophic pipeline accident involving MIC as the main cause was in Carlsbad, New Mexico, which was a natural gas pipeline explosion [7].

One of the most destructive microorganisms that are related to MIC by accelerating the corrosion process is known as sulfate reducing bacteria (SRB). Corrosion rates of 4 to 5 mm/year can be expected due to SRB activity [8]. SRB plays an important role in accelerating corrosion process in pipeline systems and it can have a severe impact on oil and gas industries. These bacteria are non-pathogenic, are anaerobic microorganisms in nature and have the ability to reduce sulphate into sulphide. SRB produces enzymes that have the power to accelerate the reduction of sulfate compounds to corrosive hydrogen sulphide (H2S). SRB influences the initiation of corrosion and oxidizes the organic matter or hydrogen

for energy source [9, 10]. In other words, SRB acts as a catalyst in reduction reaction. SRB related with strain Desulfovibrio does not require a high nutrient medium; in fact it can make do with a fairly simple mineral medium. Using hydrogen (H2) as an electron donor does not leave organic compounds as a by-product of decontamination [11].

Chemical biocides, comprised of different oxidizing and non-oxidizing agents, for example glutaraldehyde and benzalkonium chloride, are most commonly use in practice to treat MIC [12, 13]. However, the use of chemical biocides techniques may pollute the environment. In addition, it is costly as there are long pipelines to be treated. Biocides also contribute to health problem [14]. Moreover, microorganism might undergo mutation and resistance to biocides after prolonged used. One of the ecological strategies that are proposed to treat microorganism, for example, SRB is by utilizing UV radiation [15, 16]. Ultraviolet (UV) light is the portion of the electromagnetic spectrum that lies between X-rays and visible light. Generally, UV rays are usually divided into four different wavebands, vacuum UV between 100 and 200 nm, UV-C between 200 and 280 nm, UV-B between 280 and 315 nm, and UV-A between 315 and 400 nm [17]. For the best result, it is recommended to use UV radiation with wavelength of 254 nm which is in the germicidal UV-C spectrum. The UV radiation kills the microbial cells primarily due to its destructive effects on deoxyribonucleic acid (DNA) [18]. Ultraviolet light is absorbed by a double bond in pyrimidine bases (such as thymine and cytosine in DNA), opening the bond and allowing it to react with neighbouring molecules. If it is next to a second pyrimidine base, the UV-modified base will form direct covalent bonds with it. The most common reaction forms two new bonds between the neighbouring bases, which forms a tight four-membered ring [19]. In this paper, experimental works have been carried out to explore the potential of UV radiation to inhibit SRB growth. These approaches can be an alternative benign technique in replacing chemical biocides to inhibit SRB activity.

## 2. Experimental Investigations

### 2.1. Microorganism

The SRB samples were obtained from local pipeline operator which had been isolated and underwent polymerase chain reaction (PCR) to obtain sequence code. Based on Molecular Evolutionary Genetics Analysis (MEGA 5.2.2), the SRB at Sg. Ular site was identified as *Desulfovibrio sp* (99% similarity) strain. The origin of the bacteria is near the river and mangrove forest with the coordinate of 4°2'33.10" N, 103°23'0.73" E in the East Coast of Malaysia. In addition, the soil condition of Sungai Ular is swampy and consists of clay and fine sand. Based on previous research [20, 21], the metal loss rate at Sungai Ular site were substantially higher compared to other sites, due to presence of SRB.

### 2.2. Cultivation medium

Postgate medium C was used as the selective cultivation medium for the SRB with chemical composition as shown in Table 1. The pH of the cultivation medium was adjusted using buffer solution to 7.5 and was incubated at temperature of 37˚C.

**Table 1. Chemical composition of Postgate C medium for 1000 ml.**

| Component | Weight [g] |
|---|---|
| Sodium Lactate | 6.000 |
| Sodium sulfate ($Na_2SO_4$) | 4.500 |
| Ammonium chloride ($NH_4Cl$) | 1.000 |
| Yeast extract | 1.000 |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | 0.500 |
| Sodium citrate.$2H_2O$ ($C_6H_5Na_3O_7.2H_2O$) | 0.300 |
| Calcium chloride.$6H_2O$ ($CaCl_2.6H_2O$) | 0.060 |
| Magnesium sulphate. $7H_2O$ ($MgSO_4.7H_2O$) | 0.060 |
| Iron (II) sulfate. $7H_2O$ ($FeSO_4.7H_2O$) | 0.004 |

## 2.3. Coupon material

Corrosion specimen material used was a carbon steel coupon grade API 5L X-70 which was obtained from local pipeline operator. The composition of the carbon steel is as follows: 97.093% Fe, 0.078% C, 1.67% Mn, 0.15% Ni, 0.012% P, 0.3% Si, 0.023% Cu, 0.275% Cr, 0.11% Ti, and 0.002% S. [22]. Steel samples were cut into 10 mm x 20 mm x 5 mm dimension to fit into the anaerobic vials openings. The test coupons were polished with 100 grit Si-C paper and dried with acetone (99.5% purity) to remove all form of dirt, grease and small Si-C particles on the corrosion specimen surface. Figure 1 shows the cleaned and dried specimens were coated with prime coat leaving only the top surface exposed. The specimens were then dried overnight. Prior to use, the exposed surface area of the specimen was polished with series of Si-C paper grade (320, 600 and 800), followed by acetone (99.5% purity) degreasing.

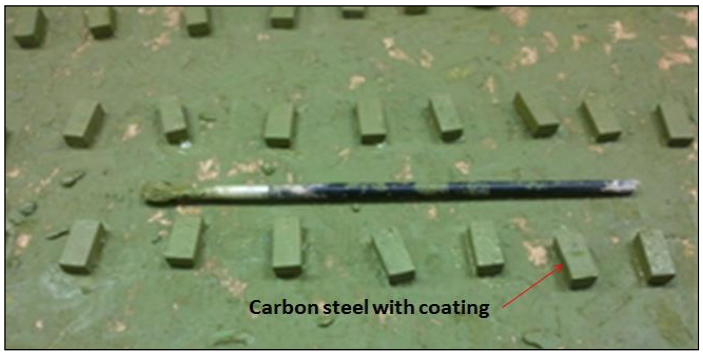


**Fig. 1. Coating the steel coupons with prime coat.**

## 2.4. Method of inoculation

Chemical compositions listed in Table 1 were mixed with one liter of distilled water. The pH was adjusted by using buffer solution to the desired pH and the medium was sterilized in an autoclaved at 121°C for about 15-20 minutes. In order to maintain the anaerobic condition, oxygen-free nitrogen gas was sparged in the medium while the medium was still warm. The test coupons and 100 ml of

medium were then transferred into anaerobic vials and sealed with butyl rubber to maintain the anaerobic condition. 2 ml of SRB seed (2% from medium amount) was inoculated into the 100 ml medium and incubated at temperature 37˚C. Within 2 to 3 days, the presence of SRB was indicated with an existence of black color and rotten egg smell of the medium.

## 2.5. Testing and disinfection process

In metal loss test and disinfection process, carbon steel specimens were retrieved from anaerobic vials based on a weekly basis up to 21 days (day 7, 14, and 21). The exposed surface of corrosion specimens were cleaned with Clarke's solution in order to remove all forms of dirt as shown in Fig. 2.

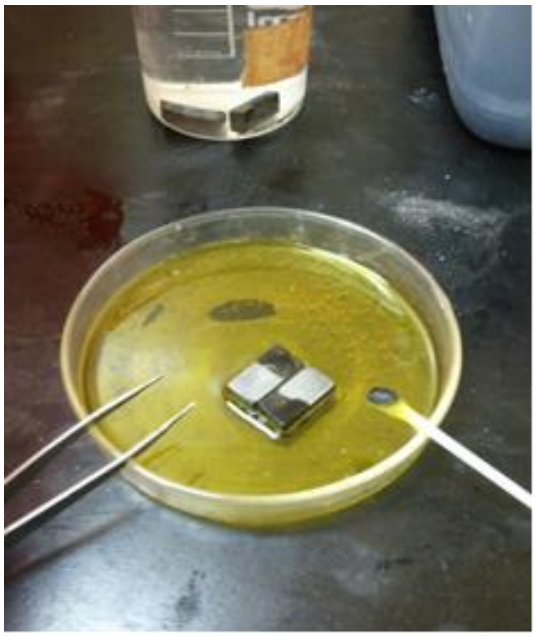

**Fig. 2. Cleaning process of steel coupons with cleaning reagent.**

The corrosion specimen were dried and their weight before and after immersion in the medium was recorded. Metal loss was determined by using Eq. (1) [20, 21] where $W_o$ is the initial weight of coupon and Wa is the final weight of coupon in gram (g).

$$\text{Metal loss} = \frac{Wa - Wo}{Wo} x100\% \quad (1)$$

## 2.6. Disinfection of SRB using ultraviolet radiation

First, before disinfection processes the optimum growth parameter pH (4.5, 5.5, 6.5, 7.5, 8.5, and 9.5) and temperature (20, 37, and 60˚C) test for SRB growth was

conducted. By observing the growth of SRB using turbidity method, the optimum pH and temperature for the growth of SRB was 8.5 and 37 °C respectively. Second, the corrosion specimen and cultivation medium with optimum pH were transferred into the anaerobic vials. Then the filled anaerobic vials were exposed to UV radiation at wavelength of 254 nm (contact time was 60 minutes).

The exposed sample was left incubated at temperature 37 °C for 21 days after the treatment process. Turbidity measurement is one of the enumeration methods for microorganism population. Nevertheless, turbidity measurement method is lacking in its capability to measure the exact number of microorganisms present in the solution or medium since the presence of microorganism is represented by the cloudiness or haziness of the medium. In this study, the turbidity of the medium with SRB was taken based on a daily basis until day 21 using spectrophotometer (wavelength 600 nm) HACH DR 4000. Serial dilution method was performed to ease the measurement of turbidity of the growth medium which contains SRB.

## 3. Results and Discussion

In this study the corrosion specimens for experimental work are divided into three categories; sample not exposed to SRB and UV radiation (named as control sample), sample exposed to SRB without UV disinfection (named as untreated sample) and the third sample which is exposed to SRB with UV disinfection (named as treated sample). After the immersion periods, corrosion specimens were removed and cleaned with Clarke's solution and dried before weighing. Final weights of the steel coupons were taken to calculate the weight losses. Data of weight loss in this study represent an average data in several testing. The percentage of metal loss was elucidated clearly in Fig. 3 where 0.221, 0.342 and 0.400 % for untreated sample and 0.192, 0.269 and 0.366 % for control sample at day 7, 14 and 21 respectively.

Figure 3 indicates the weight loss over time. The pH and temperature were adjusted at 8.5 and 37 °C, respectively. From the pattern of the graph we can see that the metal loss increase over time for both samples. Each point in the line graph showed the average data of four steel coupon specimens with standard deviation error-bar plot. The rate of metal loss (slope gradient) seems to increase in the first 7 days and then begin to decline thereafter due to depletion of nutrients for the microorganism to survive for untreated sample. Our finding also revealed that the metal loss rate for corrosion specimen in control sample was quite low as compared to the specimens that were immersed (untreated sample) in SRB seeds. Theoretically, when bacteria are attached into metal surface, a thin film known as biofilm starts to form [23]. There are three categories of significant impact when the biofilm forms onto surface known as i) production of differential aeration or chemical concentration cells, ii) production of organic and inorganic acids as metabolic by-products and also iii) production of sulfides under oxygen free condition [24]. The formations of biofilm start to change the electrochemical properties as well as physical properties of the metal [25].

The presence of sulfate ions in the medium influenced the formation of biotically generated sulfides in the corrosion products [26]. The biofilm layer thickens as the metal surface is prolonged to react with microorganisms. This

occurrence will cause increasing amount of metal loss and contribute to pitting corrosion dependent on time. In order to investigate the performance of UV disinfection upon SRB growth, turbidity of the treated and untreated samples were recorded on selected days. Higher turbidity value indicated the higher population of active SRB presence. The finding is consistent with findings of past studies by Postgate (1984), Davidova (2001) and Abdullah (2014) which discovered the characteristic of rotten egg smell (hydrogen sulfide, $H_2S$) and black coloured solution were evidence of SRB activity and metabolism in the medium [27, 28, 29]. Present study showed that turbidity method could be one of the procedures for SRB enumeration. Theoretically, when the turbidity (or cloudiness) of a sample increases, the number of cells per millilitre will also increase in the culture [30]. Figure 4 clearly illustrates the average data in duplicate tests with standard deviation error-bar plot for UV radiation disinfection effectiveness upon *Desulfovibrio sp* strain. Our finding revealed that disinfection of SRB growth by using UV radiation proved to be effective, since the turbidity value declined after the treatment.

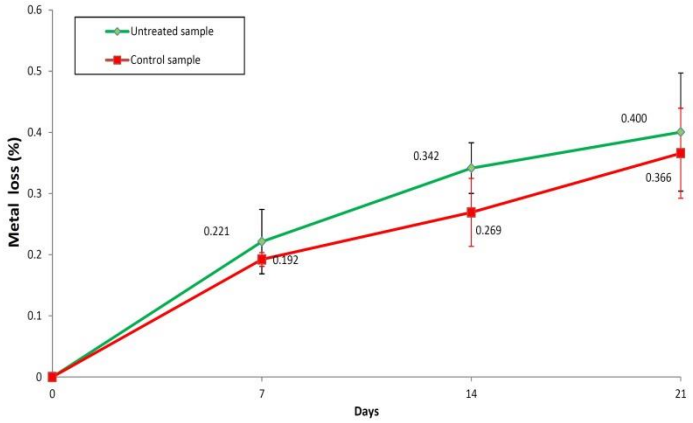


**Fig. 3. Graph of metal loss (%) versus day.**

From the graph pattern in Fig. 4, it is apparent that the treatment efficiency increase over time. The results show that, at the early stage of disinfection period the turbidity value of treated sample (1.141) was slightly higher compared to untreated sample (1.135) at day 2. In addition, the turbidity value of treated sample (0.771) was much lower compared to untreated sample (0.846) over time at day 21. Among the plausible explanations for these findings is that when samples are exposed to the UV radiation, the cellular DNA of microorganism absorbed the energy from the UV light, causing adjacent thymine molecules to undergo dimerization [31]. This process would result in the replication of the chromosome before binary fission is impaired, leaving the bacteria unable to produce proteins or reproduce which ultimately leads to the death of the microorganisms [32] and decreased numbers of active microorganism in the sample. What is interesting in this data is that, the longer the sample are exposed to UV radiation, the more will turbidity decline due to extermination of active SRB in the sample. UV radiation with effective germicidal activity peaks at 254

nm is considered as the most effective to disinfect microorganism which can be well-compared with the UV wavelength used in this study [33].

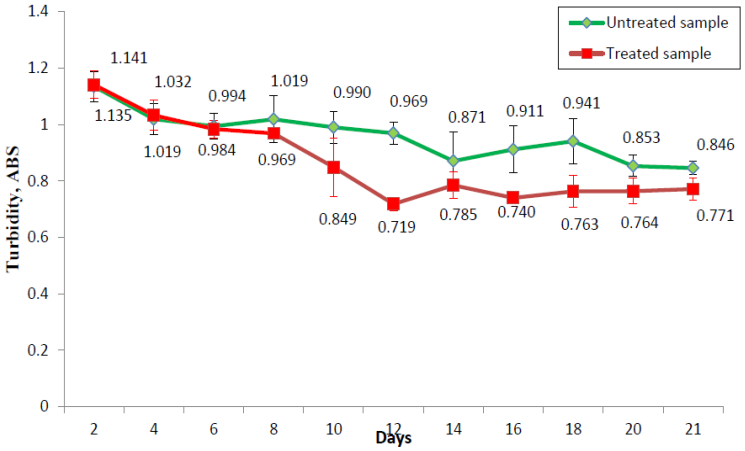


**Fig. 4. Graph of turbidity between treated and untreated sample upon UV radiation.**

## 4. Conclusions

Based on the result, it is reasonable to indicate that SRB can cause serious corrosion damage on the carbon steel coupon API 5L-X70 over time. The findings also provided evidence that metal loss increased linearly over time upon exposure to SRB activity. Low turbidity values for treated sample when compared to untreated sample also provided evidence that UV radiation with wavelength 254 nm will inhibit SRB growth and activity. Thus, this could be strong evidence for UV radiation to be utilised side-by-side with chemical biocides in the efforts to mitigate MIC. Hence, reduce the consumption of chemical substances during pipeline maintenance. Further research on this topic might pave a way for a total elimination of chemical biocides in the pipeline maintenance program.

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## References

1. Hopkins, P.; and Associates, A.P. (2000). Pipeline integrity reviews. *Proceedings of International Pipeline Conference*, 44, Morelia, Mexico, 1-15.
2. Shaw, B.A.; and Kelly, R.G. (2006). *The electrochemical Society Interface: What is Corrosion. Spring, 24-26.*
3. Peng, C.G.; and Park, J.K. (1994). Principal factors affecting micro-biologically influenced corrosion of carbon steel. *Corrosion*, 20(9), 669-675.

4. Zarasvand, K.A.; and Rai, V.R. (2014). Microorganisms: Induction and inihibition of corrosion in metals. *International Biodeterioration and Biodegradation*, 87, 66-74.
5. Ben, H.T.; Glenn, M.L.; James F.D.; Elizabeth, T.; Fengmei S.; Carl, F. P.; Kent, E.C.; and Richard, A. (2010). Corrosion Control In Oil And Gas Pipelines. *Pipeline and Gas Journal*, 237(3), 1-2.
6. Yang H.; Xue, W.P.; Fu, Z.X.; Wang, W.; and Song, K. (2010). Research Advances on Microbiologically Influenced Corrosion and Its Prevention Measures in Marine Environment. *Chemistry and Bioengineering*, 27, 1-5.
7. Sooknah, R.; Papavinasam, S.; and Revie, R.W. (2008) Validation of a predictive model for microbiologically influenced corrosion. *Proceedings of the Corrosion Conference and Expo (CORROSION'08)*, NACE International, Houston, Tex, USA.
8. Javaherdashti, R. (2012). The Basics of Microbial Corrosion: An industrial Approach, conjunction with 25th Anniversary of Institute of Material Malaysia (IMM). *8th International Materials Technology Conference and Exhibition (IMTCE)*, Kuala Lumpur, Malaysia, 1-25.
9. Ruwisch, R.C.; Kleinitz, W.; and Widdel, F. (1987). Sulfate reducing bacteria and their activities in oil production. *Journal of Petroleum Technology*, 97–106.
10. Zhang, C.; Wen, F.; and Cao, Y. (2011). Progress in research of corrosion and protection by sulfate reducing bacteria. *Procedia Environmental Sciences*, 10, 1177–1182.
11. Caffrey, S.; Hyung, S.P.; Voordouw, J.K.; and Zhili, H. (2007). Function of periplasmic hydrogenases in the sulfate reducing bacterium Desulfovibrio vulgaris Hildenborough. *Journal of Bacteriology*, 6159-6167.
12. Zhu, Z.Y.; Modi, H.; and Kilbane, J.J. (2006). Efficacy and risks of nitrate application for the mitigation of SRB induced corrosion. *CORROSION NACExpo 2006*. 1–41. Houston: NACE International.
13. Street, C.N.; and Gibbs, A.J. (2010). Eradication of corrosion causing bacterial strain Desulfovibrio vulgaris and Desulfuricans using photodisinfection. *Northern Area Western Conference*. Calgary, 1–14.
14. Jhobalia, C.M.; Hu, A.; Gu, T.; and Nesic, S. (2005). Biochemical engineering approaches to MIC. *Corrosion 2005*. Houston, 1–12.
15. Wang, J.; Yang, F.; Yuan, X.; Liu, B.; Wu, H.; and Sui, X. (2005). Successfully sterilizing the sulfate bacteria with ultraviolet radiation in produced water treatment in Daqing oilfield. *Asia Pacific Oil & Gas Conference and Exhibition.* Jakarta, Indonesia, 1–5.
16. Bjorndalen, N.; Mustafiz, S.; Tango, M.; and Islam, M. (2003). A novel technique for prevention of microbial corrosion. *Energy Sources*, 25, 945.
17. Meulemans, C.C.E. (1986). *The basic principles of UV-sterilization of water. In:Ozone and Ultraviolet water treatment*, Aquatec Amsterdam, Paris: International Ozone Association, 1986: B.1.1-B.1.13.
18. Haggstrom, J.A.; Weaver, J.D.; Gloe, L.M.; and Holtsclaw, J. (2011). *UV Light Treatment Methods And System*. United States: United States Patent Application Publication.

19. Lawal, O.; Shannon, K..; Gloe, L.; King, K.; Warren, W.; Hargy, T.; and Fong, F. (2010). Ultraviolet disinfection effectively controls oilfield sulfate reducing bacteria. *IUVA News*, 12(3), 17–20.
20. Yahaya, N.; N.M. Noor.; Othman S.R.; Sing, L.K.; and Din, M.M. (2011). New technique for studying soil-corrosion of underground pipeline. *Journal of Applied Science*, 11, 1510-1518.
21. Norhazilan, M.N.; Nordin, Y.; Lim, K.S.; Siti, R.O.; Safuan, A.R.A.; and Norhamimi, M.H. (2012). Relationship between soil properties and corrosion of carbon steel. *Journal of Applied Science Research*, 8, 1739-1747.
22. Norhazilan, M.N.; Yahaya, N.; Abdullah, A.; Tahir, M.M.; and Sing, L. K. (2012). "Microbiologically influenced corrosion of X-70 carbon steel by Desulfovibrio vulgaris," *Advanced Science Letters*, 13, 312-316.
23. Korbin, G. (1993). A Practical Manual on Microbiologically Influenced Corrosion, 1(2), *NACE International*, 233.
24. Javaherdashti, R. (2004). Modelling microbiologically influenced corrosion of N-80 carbon steel by Fuzzy Calculus. *Metallurgical and Materials Transaction. A*, 35, 2051-2056.
25. Javaherdashti, R. (2011). Impact of sulphate reducing bacteria on the performance of engineering materials. *Applied Microbial. Biotechnol.*, 19, 1507-1517.
26. Alabbas, F.M.; Kakpovbia A.; Mishra B.; Gavanluei A.B.; Spear J.R.; and Olson D.L. (2012). Effects of sulfate reducing bacteria on the corrosion of X-65 pipeline carbon steel. NACE 2012-1140: *Proceedings of CORROSION 2012*, Salt Lake City, Utah, March 11-15.
27. Postgate. J.R., (1984). *The Sulphate-Reducing Bacteria*, Cambridge U.Press, London.
28. Davidova I., Hicks M.S., Fedorak P.M., and Suflita J.M. (2001). The influence of nitrate on microbial processes in oil industry production waters. *Journal of Industrial Microbiology and Biotechnology*, 27, 80.
29. Abdullah, A.; Yahaya, N.; Noor, N.; and Rasol, R. M. (2014). Microbial corrosion of API 5L X-70 carbon steel by ATCC 7757 and consortium of sulfate reducing bacteria. *Journal of Chemistry*, 1–7.
30. Michelle F. (2007). *Bacterial enumeration and dilutions lab*. Lab written in Microbiology for health science lab 5. 1-8.
31. Crane, B.; Neal, G.; and Warren, W. (2011). Improved process provides more effective Ultraviolet light disinfection of fracturing fluids. *Proceedings of SPE Americas E&P Health, Safety, and Environmental Conference*. Houston, 1–9.
32. Pommerville; and Jefferey. (2001). *Fundamentals of microbiology*, ($7^{th}$ ed.). Sudbury: Jones and Bartlett Publishers.
33. Wang, J.; Yang, F.; Yuan, X.; Liu, B.; Wu, H.; and Sui, X. (2005). Successfully sterilizing the sulfate bacteria with Ultraviolet radiation in produced water treatment in Daqing Oilfield. In *Asia Pacific Oil & Gas Conference and Exhibition*, Jakarta, Indonesia, 1–5.