Sensitivity of Sulphate Reducing Bacteria to Oilfield Biocides using a Rapid Detectable Culture Media Method

Ohimor, Evuensiri Onoghwarite¹, Ononiwu, Prosper Ikechukwu² and Ideh, Oghenejavwaire³

¹Federal University of Petroleum Resources, Effurun, Delta State, Nigeria, Phone No: +2348033888418: E-mail address: ohimor.evuensiri@fupre.edu.ng

²Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, Phone No: +2347036331364: E-mail address: prince3iyke75@gmail.com

³Federal University of Petroleum Resources, Effurun, Delta State, Nigeria, Phone No: +2348142764259: E-mail address: Idehwaire@yahoo.com

ABSTRACT

Sulphate-reducing bacteria (SRB) are the most dominant bacteria that lead to Microbiologically Induced Corrosion (MIC), which is one of the main practical concerns in the sustenance and management of pipelines in the petroleum industry. These bacteria are anaerobic and non-pathogenic but powerful enough to cause problem both economically and ecologically considering the large amounts of toxic hydrogen sulfide they generate in aquatic ecosystems. Thus, the study is aimed at comparing the sensitivity of SRB in oilfield produce water to some commercial biocides, Tetrakis hydroxymethyl phosphonium sulphate (THPS) and Gluteraldehyde (GLUT) by using Turbidity Method in measuring the effect of the biocides on microorganism. The method entails determining the Minimal Inhibitory Concentration (MIC), which is just sufficient to prevent rapid growth in a very sensitive and suitable medium. This method, instead of noting ferrous sulphide production, it measures changes in optical density and sulphate reduction of a logarithmic culture of the test strain exposed to the biocide. Inhibition of SRB growth did not occur at a concentration of 50ppm for THPS biocide but was observed for glutaraldehyde biocide. The effect of a biocide on a cell is normally dependant on its concentration and it can be seen from the slight increase and levelling off of $\Delta 600$ at 50ppm that glutaraldehyde is bacteriostatic at this concentration while THPS was observed to be bacteriostatic at 100ppm. The biocidal activity and biocidal efficiencies and recorded that up to 90% for commercial GLUT and up to 85% for commercial THPS at 100ppm.

Keywords: Sulphate-reducing bacteria, culture media, Biocide, Corrosion, MPN

Corresponding Author: Ohimor E. O.

INTRODUCTION

Previous studies agree that sulphate-reducing bacteria are the most dominant bacteria that lead to Microbiologically Induced Corrosion (MIC) [1, 2, 3]. Microbiologically Induced Corrosion (MIC) is one of the main practical concerns in sustaining and management of pipelines in the petroleum industry [4]. Sulphate-reducing bacteria are anaerobic and non-pathogenic bacteria, however powerful enough to cause problem both economically and ecologically [5] considering the large amounts of toxic hydrogen sulfide they generate in aquatic ecosystems.

The activities of SRB in natural and artificial systems are of great concern also to engineers in many other different industrial operations [6, 7, 8,]. Oil industries are seriously affected by the sulfide generated by SRB [9, 10, 1, 11]. Activities in the oil and gas production involve

the extraction of large amounts of water which are often injected back into the reservoir; secondary recovery, to maintain reservoir pressure as high as possible. During this operation, one of the main steps consists of biocide treatment against sulphate reducing bacteria (SRB) which is the well-known microorganism within the oilfield whose metabolic (respiration) product is H_2S [12].

In efforts to reduce the risk of formation damage and corrosion downhole, the industry uses biocides to control bacteria levels that can contribute to these damages. There are several types of biocide chemical commercially available for oil and gas application; the most common types used are Tetrakis Hydroxymethyl Phosphonium Sulphate (THPS) and Gluteraldehyde (GLUT). A favoured benefit to THPS is that it is considered degradable and non-bioaccumulative in the environment. Therefore it is extremely important to evaluate biocide chemicals prior to start-up of any system [13]. Produced water is largely seen as contaminated water. And as such, considering that biocides generally inhibit the growth of SRBs, this study tries to compare the performance of two different commercial biocides in reducing the contamination of the produced water.

One of the simplest ways to measure the effect of a biocide on microorganism is by determining the Minimal Inhibitory Concentration (MIC) which just prevents growth in a suitable medium. Thus, the present study is aimed to compare the sensitivity of SRB from oilfield produce water to commercial biocides (THPS and GLUT) using the turbidity method.

2. MATERIALS AND METHODS

2.1 Culture Medium (Postgate C Medium)

Postgate C medium was prepared according to the chemical composition shown in Table 1. All the ingredients were mixed together with 1litre of distilled water and stirred for 30 minutes. The pH was adjusted to 7.5 before autoclaved at $121^\circ C$ for 15 minutes. The medium was then sparged with nitrogen gas for approximately one hour to remove oxygen from it.

Table 1: Composition of Postgate C bacterial medium (Postgate, 1984)

S/N	Ingredients	Concentration (g/l)
1	Sodium Lactate	6.0
2	Na_2SO_4	4.5
3	NH_4Cl	1.0
4	Yeast Extract	1.0
5	KH_2PO_4	0.5
6	$C_6H_5Na_3O_7 \cdot 2H_2O$	0.3
7	$CaCl_2 \cdot 6H_2O$	0.06
8	$MgSO_4 \cdot 7H_2O$	0.06
9	$FeSO_4 \cdot 7H_2O$	0.004

2.2 Physicochemical Analyses of the Produced Water

Physicochemical parameters such as pH, electrical conductivity, salinity, total petroleum hydrocarbon (TPH), sulphate concentration were determined using pH meter, EC1 meter (Accepta Boiler Water Test Kit) Metrohm Titrator, Fourier transform Infrared Spectrometer and Sensidyne Nephelometer respectively. Determination of other chemical parameters of Produce water: Such as Total Dissolved Solids, Hardness, Salinity, Chlorides, Sulphite, Phosphate, Electrical Conductivity, Alkalinity, were done according to the individual procedures from Accepta Boiler Water Test Kit Manual.

2.3. Enumeration of SRB using Most Probable Number (MPN)

The produced water was inoculated into a serum bottle containing 9ml of Postgate C medium, the bottle was incubated at 30°C for 2 days. After SRB growth was observed in the medium, 1ml of the bacterial growth was inoculated into a serum bottle containing Postgate C medium. The number of bacteria present in 1ml was determined by triplicate extinction serial dilution; 12 serum bottles were arranged in sets of 3, A, B, C of four (4) successive dilutions (3x4). The bottles were incubated for 10 days and the numbers of bottles with positive growth were counted and the MPN of microorganisms was determined using the MPN table for a three-replicate design from the Food and Drugs Administration Bacterial Analytical Manual.

2.4. Biocide Test Procedure

The freshly prepared Postgate C medium was dispensed into (4) 10ml serum bottles. 0.1ml of the produced water was inoculated into the 4 serum bottles containing the Postgate C medium and the bottles were incubated at 30°C for 4 days. The formation of black precipitate and a rotten egg smell in the medium indicated the presence/growth of SRB.

Fresh Postgate C medium was dispensed into 33 serum bottles, 16 of the serum bottles were inoculated with THPS biocide while another 16 bottles were inoculated with GLUT biocide at different concentrations (4 bottles each dosed at 50ppm, 100ppm, 150ppm and 200ppm) respectively and the last bottle (control) was inoculated with 1ml of distilled water. The bottles were autoclaved at 121°C for 15mins. 1ml from the serum bottles already containing SRB growth was inoculated into the 32 bottles containing the biocides at different concentrations. The effect of the biocide on SRB was determined by measuring the turbidity of culture absorbance at a wavelength of 600nm using a UV spectrophotometer at 1 day interval for 4 days. The efficiency of the biocide was calculated using the formula:

$$E\% = \frac{\text{Uninhibited} - \text{Inhibited}}{\text{Uninhibited}} \times 100\%$$

3. RESULTS AND DISCUSSION

The Physicochemical properties of produced water vary considerably depending on the geographical location of the field, the geological formation with which the produced water has been in contact for thousands of years and the type of hydrocarbon product being produced. Physicochemical analysis of produced water as recorded in table 2 revealed that it was a very good source for SRB due to the sulphate contents (1795 mg/l).

Table 2: Physicochemical properties of the produced water sample

PARAMETERS	VALUE
pH (mg/l)	6.58
Temperature (°C)	27
Alkalinity (mg/l)	Nil
BOD (mg/l)	674
Specific Gravity	1.0854
Electrical Conductivity (µs/cm)	2620.0
Resistivity (Ohm m)	0.0784
Total Dissolved Solids (mg/l)	1258.5
Hardness (mg/l)	25053.2
Total Petroleum Hydrocarbon (mg/l)	117
Total Soluble Solids (mg/l)	3348
Salinity (mg/l)	1300.6
Chlorides (mg/l)	204.50

Sulphate (mg/l)	1795.00
Phosphate (mg/l)	18.00

3.1. Enumeration of SRB from the produced water using the MPN Method.

Table 4.2 shows the MPN and average turbidity reading for both sulphate-reducing bacterial strains present in Postgate C media using the Most Probable Number (MPN) bacterial enumeration method.

Table 3: Bacterial growth observation for MPN of SRB in Produce Water

Sample: Produce Water		Test: MPN of SRB		
Treatment Dosage: Nil				
Final changes in Bottles after 28 days				
	Dilution 1 (10 ¹)	Dilution 2 (10 ²)	Dilution 3 (10 ³)	Dilution 4 (10 ⁴)
Replica 1 Bottles	+	+	+	-
Replica 2 Bottles	+	+	+	-
Replica 3 Bottles	+	+	-	-
Counts	skipped	3	2	0
NB: '+' means growth and '-' means no growth Colour change: Light yellow/Beige to Black				

The results below were calculated from the Most Probable Number table using the findings in the tables above;

Table 4: MPN of SRB in Produce Water

Water Sample	MPN of Bacterial SRB (bacterial/ml)	Turbidity (Absorbance)
Produce Water	9.5×10^3	0.419

Figure.1: Evidence of SRB Growth and Metabolism in the Postgate's C Bacterial media

3.2. Turbidity of Produce water treatment with Biocide

The Figures 4.2 and 4.4 show the average turbidity reading for sulphate-reducing bacterial strains present in the produced water treatment with THPS and GLUT biocide at different treatment rates over different time interval and the temperature set for growth was 30°C throughout the experiment. In order to get reliable growth pattern of SRB, turbidity test was on a daily basis for a period of 4 days.

In Figure 4.3, black colour of the medium and rotten egg smell is the indicator of the SRB growth within 1 to 3 days after the injection of SRB seed into the THPS treated medium, which was observed in the treatment dosage of 50ppm (Figure 4.3). The bacterostatic and

bactericidal activities of the biocide THPS on the SRB was confirmed by the decreased number of turbidity over time for the different treatment dosages of 100ppm, 150ppm and 200ppm of the biocide.

The bactericidal activity identified in the graph showed a drastic decreasing pattern of the turbidity figures recorded in treatment rate of 100ppm to 200ppm of the THPS biocide. On the final day of the experiment, the turbidity in the bacterial media recorded the lowest at 0.197, 0.029, 0.013 and 0.012 Abs for 50ppm, 100ppm, 150ppm and 200ppm respectively.

The bactericidal activity of the biocide GLUT on the SRB was confirmed by the drastic decreased number of turbidity over time for the different treatment dosages of the biocide (Figure 4.4). Inhibition of SRB growth occurred at a concentration of 50ppm. On the final day of the experiment, the turbidity in the bacterial media recorded the lowest at 0.029, 0.019, 0.008 and 0.004 Abs for 50ppm, 100ppm, 150ppm and 200ppm respectively.

Figure 2: Activity of THPS Biocide on the SRB at different treatment rates with different time interval

Figure 3: Bacterostatic and bactericidal activities of the biocide THPS on the SRB at different treatment rates and different time interval after 4 days incubation.

Figure 4: Activity of GLUT Biocide on the SRB at different treatment rates with different time interval

Figure 5: Bacterostatic and bactericidal activities of the biocide GLUT on the SRB at different treatment rates and different time interval after 4 days incubation.

3.3. The Efficiencies (E %) of Biocides against SRB

Table 5: Efficiency (%) of the THPS biocides against SRB

Treatment (ppm)	Rate/Dosage	Time Intervals (Day)				
		0	1	2	3	4
50		0	-3	-4	-4	-3
100		0	32	44	62	85
150		0	45	57	91	93
200		0	47	59	89	94
Control		0	0	0	0	0

Table 6: Efficiency (%) of the GLUT biocides against SRB

Treatment (ppm)	Rate/Dosage	Time Intervals (Day)				
		0	1	2	3	4
50		0	41	50	62	85
100		0	45	50	67	90
150		0	61	83	96	96
200		0	63	85	96	98
Control		0	0	0	0	0

3.4. DISCUSSION

Physicochemical analysis of the sampled produce water as recorded in table 2 revealed that it was a very good source for SRB due to the sulphate contents (1795mg/l).

SRB are present in most soils and water, but are outnumbered by other types of microbes except in special environments. Consequently, enrichment of the needed environments with these bacteria is usually necessary before isolation [14] and enumeration is attempted. On the other hand the presence of reducing agent in culture medium makes isolation a much less formidable task [15]. Postgate medium C is a multipurpose medium for detecting and culturing strains of Sulphate Reducing Bacteria (SRB), including *Desulfovibrio* and *Desulfotomaculum*. This medium consists of different chemical composition and the results interpreted that the chemical composition in the medium enhances the growth of SRB. Moreover, the medium has a carbon source, which is lactic acid and highly recommended for the cultivation of SRB, while the presence of iron (Ferrum) in the medium is an essential component which is responsible for assisting the microbial activity of SRB. Dennis and Julia [16] stated that iron sulphides (FeS) are one of the main characteristic products related to MIC. In a way to reduce the delay that is inherent in the API testing methods we have adopted a simple and rapid technique for studying the effect of a biocide on SRB by measuring the inhibition of SRB and Sulphate reduction, thus, the turbidity method developed by Sharma *et al.* [17].

Tentatively, broth will become turbid due to the presence and growth of SRB in liquid culture [18]. The more turbid is the medium, the higher number of SRB present in the medium. Postgate [15] stated that the characteristic of unpleasant rotten egg smell from hydrogen sulphide and black coloured solution are mainly due to the evidence of SRB growth and its metabolism in the medium. Therefore, the turbidity measurement gives enough quantitative information which is needed in predicting the growth of SRB. The precipitate in medium C aids growth of tactophilic strains. This medium is recommended for long term storage of strain. So for diagnostic purposes media often prescribed are those which contain about 0.5% of a ferrous salt. This forms a black precipitate of FeS when sulfide is formed, so blacking of the medium as a whole, or the zone round a colony, is an evidence for bacterial sulfate reduction [19].

Table 4 shows the MPN and average turbidity reading for sulphate-reducing bacterial strains present in Postgate C media using the Most Probable Number (MPN) bacterial enumeration method.

Biocide treatment rate of 50ppm were used as lower range as this interval is recommended for biocides with potential off-shore applications [20]. Inhibition of SRB growth did not occurred at a concentration of 50ppm for THPS biocide (Fig 3) but was observed for glutaraldehyde biocide (Fig 5). The effect of a biocide on a cell is normally dependant on its

concentration and it can be seen from the slight increase and levelling off of $\Delta 600$ at 50ppm that glutaraldehyde is bacteriostatic at this concentration while THPS was observed to be bacteriostatic at 100ppm. However, at 100ppm the decrease in $\Delta 600$ reflects a bactericidal action with cell lysis for the glutaraldehyde.

Knowledge of the respiratory activity of SRB in the presence of an inhibitor is essential as most of their destructive properties are caused by their respiration of sulphate to sulphide. Figure 5 shows clearly that whilst 50ppm glutaraldehyde inhibits SRB growth, sulphate reduction still occurs at a reduced rate. Complete inhibition of sulphide production is only achieved at a glutaraldehyde concentration of 100ppm. SRB growth was still observed at 50ppm of THPS but at 100ppm THPS inhibits SRB growth, though sulphate reduction still occurred at a reduced rate.

Due to the economic losses as well as environmental health and safety hazards caused by the activity of stabilized mixed culture containing sulfate reducing bacteria, in many industrial sectors such as the oil and gas industry, it was important to minimize the risks resulting from SRB activity. These bacteria are mainly sulfate reducers, and their growth frequently causes severe corrosion problems in oil well pipes. One of the simplest ways to measure the effect of biocides on an organism is by determining the Minimal Inhibitory Concentration (MIC) which just prevents growth in a suitable medium. The antibacterial agent is serially diluted in the medium or applied at different treatment rates and standardized inocula of the test strain/growth are added. After incubation for a predetermined growth, the cultures are examined and the MIC for the biocide is detected [17]. The MIC of microbicides and bacteriostatic substances are usually governed by the nature of the medium in which the substance is tested and also by the size of the inocula. Iron salts can increase the apparent resistance of cultures to inhibitors, and in case of *Desulfovibrio* species the presence or absence of NaCl may influence inhibition (example a quaternary biocide) [21].

In the present work the effect of tested biocides on a cell is normally dependent on its concentration and it's can be seen from the slight increase and levelling of $\Delta 600$ at growth of SRB was depressed by 50ppm of the commercial glutaraldehyde rather than 100ppm of the THPS. The results in tables 5 and 6 showed the biocidal activity and biocidal efficiencies and recorded that up to 90% for commercial GLUT and up to 85% for commercial THPS at 100ppm. The cell membrane of microorganisms is composed of several lipids and protein layers arranged together in a specific arrangement called the bilayer (or multi-layer lipoprotein structure). The presence of the lipids as a building unit in the cell membrane attains them their hydrophobic feature [21]. The selective permeability of the lipoprotein membrane represents the main function, which control the biological reaction in the cell. Hence any factor influences that permeability causes a great damage to the microorganisms, which leads it to die.

4. CONCLUSION

This research extends our knowledge on the effects of two different commercial biocides on the SRB growth pattern, while proffering a faster means of determining SRB presence. Thus, a very rapid method for detecting SRB presence and effects of biocides on their population was adopted in this study. This is in a bid to reduce the delay that is inherent in the API testing methods. This simple and rapid technique measures the inhibition of SRB and sulphate reduction based on turbidity values of culture media. Hence, the growth of SRB was observed to be depressed significantly using 50ppm and 100ppm of the commercial GLUT and THPS respectively.

The method has significant potential for application in SRB monitoring and biocides performance evaluation in the oil and gas sector.

REFERENCES

- [1] Okabe, S., Nielsen, P.H. Jones, W.J. and Characklis, W.G. (1995). Sulfide product inhibition of *Desulfovibrio desulfuricans* in batch and continuous cultures- *Wat.Res.*, 29:175-187.
- [2] Fathul K.S., Zaharah I., Adibah Y. And Madzlan A. (2008). *Isolation and Identification of Marine Sulphahte-Reducing Bacteria, Desulfovibrio sp. And Citrobacterfreundii from Pasir Gudang, Malaysia*. *Sains Malaysiana*. 37(4), 365-371.
- [3] Zhang C., Wen F. And Cao. (2011) *Progress in Research of Corrosion and Protection by Sulphate-Reducing Bacteria*. *Procedia Environmental Sciences*, 10, 1177-1182.
- [4] Yahaya, N.; Lim, K.S.; Norhazilan, M.N.; Abdullah, A. (2011). Effects of Moisture and Clay content on Soil-Corrosion*, *Malaysian Journal of Civil Engineering*. Vol. 23, No. 1, pp. 24-32
- [5] Ghazy E.A., Mahmoud M.G., Asker M.S., Mahmoud M.N., Abo Elsoud M.M. and Abdel Sami M.E. (2011). *Cultivation and Detection of Sulphate Reducing Bacteria (SRB) in Sea Water*. *Journal of American Science*, 7(2), 604-608.
- [6] Gibson, G.R. (1990). Physiology and ecology of the sulfate reducing bacteria, *J. Appl. Bacteriol.* 69:769-797.
- [7] Odom, J.M. (1990). Industrial and environmental concerns with sulfate reducing bacteria. *ASM News*, 56:473-476.
- [8] Odom, J.M. and Singleton, R. (1992). The sulfate- reducing bacteria: contemporary perspective. New York Springer-Verlag.
- [9] Hamilton, W.A. (1994). A biocorrosion: the action of sulfate reducing bacteria. In: *Biochemistry of Microbial degradation*, C. Ratledge. Dordrecht: Kluwer academic.
- [10] Peng, C.G., Park, C.G., Park, J.K. and Patenaude, R.W. 1994. Statistics base of classification of microbial influenced corrosion in fresh water system. *Wat.Res.*28:951-959.
- [11] Cullimore, R. (2000). Practical atlas for bacterial identification. Boca Raton: CRC press.
- [12] Javier, V., Sanzo, M., Koichi, S. & Chihiro, I., 2007. Numerical Evaluation of Biocide Treatment against Sulfate Reducing Bacteria in Oilfield Water Pipelines. *Journal of the Japan Petroleum Institute*, pp. 50, (4), 208-217
- [13] Bennet, D., (2017). *Oilfield Microbiology: Effective Evaluation of Biocides Chemicals*. Jakarta, Indonesia, Society of Petroleum Engineers, pp. 2-3
- [14] Widdel F. (1988). Microbiology and ecology of sulfate and sulfur reducing bacteria. In Zehnder AJB (Ed) *Biology of anaerobic microorganisms*. New York: John Wiley and Sons, PP 469-585.
- [15] Postgate, J. R. (1984). The Sulphate reducing bacteria. Cambridge University Press, Cambridge.
- [16] Dennis E. and Julia G. (2014). *Corrosion Iron by Sulphate Reducing Bacteria: New Views of an Old Problem*. *Journal of Applied Environment Microbiol.*, Vol 80, No. 4, 1226-1236.
- [17] Sharma, A.P; Battersby N.S; Stewar, D.J. (1986). Rapid method for determining the sensitivity of sulphate reducing bacteria to biocides. *GERBAM Deluxieme Colloque International de Bacteriologie marine- CNRS, Brest 1-5 October 1984. IFRMER, ACtes de colloques* 3, PP. 617-619
- [18] Maria C. and Csaba C. (1999). *Microbiological Examination of Water and Wastewater*. CRC Press, page 193.
- [19] RzecZycka, M. and Blaszczyk M.; (2005). Growth and Activity of Sulphate-Reducing Bacteria in Media Containing Phosphogypsum and Different Sources of Carbon, *Polish Journal of Environmental studies*, 14: 891-895.

-
- [20] Crouch, B.A. (1983). Evaluation of biocides for use in North Sea oil operations. In *Biodeterioration* Vol. 5. Ed Oxle. T.A. and BARRY S., pp. 725-730. John Wiley Sons; Chichester.
- [21] Bessems, E. (1983). "Biological aspects of the assessment microbial corrosion" proceedings of the conference sponsored and organized jointly by the national physical laboratory and the metals society and held NPL Teddington on 8-11 March 1983. The metal society London.