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Growth rate and Pattern of Sulphate Reducing Bacteria in Produced Water from Niger Delta Oilfield, Nigeria

[1]Ogunkunle T. Fred, [2]Oni A. Babalola & [1]Onuh, C.Y

[1]Petroleum Engineering Department. [2]Chemical Engineering Department,
Covenant University, Ota, P.M.B 1023, Ota, Nigeria.

[1]Orcid: 0000-0001-7004-3813

Abstract

In Nigeria, it is estimated that most oilfields average water to oil ratio is about 1:1, roughly one billion barrel of produced water are annually disposed during oil field production operations, as a result, deterioration of the physicochemical and bacteriological property of the disposal site has been observed. Common produced water impurities are; carbon dioxide, hydrogen sulfide, dissolved organic materials, suspended formation materials, and finally, most of time, are oxygen deficient etc. Most routine produced water treatments does not include consideration for the growth of specific bacteria such the Sulphate reducing bacteria (SRB) which are present in most produced water and ground water. In this study, four (4) produced water samples from Niger Delta oilfields, Nigeria were treated before subjecting to SRB growth monitoring test **using serial dilution**. The test procedure was based on National Association of Corrosion Engineers (NACE) International Test Method. The growth rate (culturing) of bacteria were monitored after 28 days. The results revealed there was an average constant growth rate between 4 to 28 days, inferring an exhaustion of essential nutrients and/or accumulation of toxic products of metabolism that generally limits the period of high growth.

Therefore, prior to disposal of produced water into the environment, routine produced water treatment methods should include disinfection to take care of sulphate reducing bacteria. Additionally, the presence of SRB in produced water could equally influence the on-set and propagation of biologically induced corrosion.

Keywords: Sulphate-reducing bacteria; Serial Dilution; Produced Water; Corrosion

INTRODUCTION

Generally, in subsurface rock formations water and hydrocarbon are naturally present at different saturation level (i.e. oil, gas and water, or some combination of these fluids). Most hydrocarbon reservoir rocks are initially saturated with water before the migration of hydrocarbon into such structures and subsequent trapping (Amyx & Whiting, 1960). The water in reservoir rock can come from different sources or combination of these outlets; the water could come from zones directly above or below the pay zone (hydrocarbon zone), at times it could be flow from within the pay zone, or as a result of fluids introduced during production enhancement (injection fluid or additives. This formation water becomes produced via production of hydrocarbon towards the later part of the producing life of an oil well. According to Stephenson (1992), produced water are water co-existing in the reservoir with hydrocarbon and are produced to the surface together with the hydrocarbon or comes out of the production streams upon change in reservoir conditions to surface conditions. When these water is brought to the surface, its composition is a function of the type of hydrocarbon been produced alongside with it.

Water produced alongside hydrocarbon practically comes with impurities, and depending on the concentration, our environment can be negatively impacted by the presence of these impurities in produced water. Common produced water impurities are; carbon dioxide, hydrogen sulfide, dissolved organic materials, suspended formation materials, and finally, most of time are oxygen deficient etc (Stephenson, 1992). Produced water at times comes with minute concentration of naturally occurring radioactive materials (NORM) (Gray, 1993)**.** Apart from naturally occurring impurities, the addition of oilfield chemicals as additives during production enhancement and well intervention can also introduce impurities that are not naturally found in formation water, these chemicals are; biocides, coagulants, paraffin control agents, emulsion breakers, corrosion inhibitors and scale inhibitors. The highest volume of waste in the upstream petroleum industry comes from produced water. In mature oil fields, the ratio of produced water to produced hydrocarbon can be extremely high. In Nigeria, it is estimated that most oilfields average water to oil ratio is about 1:1, roughly one billion barrel of produced water are annually disposed during oil field production operations (Chikwe & Okwa, 2016). Numerous authors discussed the growth of sulphate reducing bacteria and bacteria growth rate in stagnant seawater, this include; Wilkinson(1983) observed that the growth rate of SRB in stagnant seawater and the production of H_2S was 10 times greater when oil was present to provide carbon. Stott, (1986)

carried out detailed studies on growth conditions, sulphate reduction and other physiological processes of a large number of mesophilic sulphate-reducing bacteria (m-SRB) isolated in the 'traditional' manner from the North (Seazobell,2007) claimed that he had isolated SRB from an oil-bearing reservoir that would grow at 104ºC under a pressure of 1000atm.

In this study, the growth rate and pattern of sulphate reducing bacteria in produced water from Niger Delta oilfield was investigated. Sulphate reducing bacterial uses sulphate ions present in produced water as aceptor of electrons in oxidizing organic matter.

MATERIALS AND METHODS

The water samples were taken from four (4) different wells in the Niger Delta area of Nigeria. The samples were treated before been subjected to Sulphate reducing bacteria growth monitoring test **using serial dilution**.

Serial Dilution Analysis

This test procedure is based on NACE International Test Method. This method presents a procedure for growth pattern determined from a triplicate. Most probable number test was used to enumerate the number of bacteria per mil of the sample for a given period. The method is applicable for the media triplicate most probable number (MPN) result performed in 9ml dilutions.

1. A pack of vials containing 12 vials was taken. Labelled on each pack is the sample location, with date of inoculation and time of inoculation. The top of the pack is divided into four sections as follows; 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 3c and 4a, 4b, 4c. Using a new syringe, 1ml of the sample to be analysed was picked, injected it into 1a, shaken, using the same syringe, 1ml of the sample to be analysed was picked and inject it into 1b, and then shaken. The above steps were repeated for 1c, 2a, 2b and 2c shaken and then disposed.
2. Same was done for 3a, 3b and 3c as well as 4a, 4b and 4c. For sulphate reducing bacteria, incubation was done at 36.6ºC (98℉) for 28days, while for acid producing bacteria, incubation was done for 12days at same temperature. The pattern of positive broth bottles is then used to determine the Most Probable Number (MPN) of bacteria in the original sample using a statistically derived table.
3. A 12 by 28 table monitoring sheet was generated to monitor the growth of the bacteria daily for 28 days by indicating positive sign (+) if any bottle changes its colour to black (indicating growth) and a negative sign (-) if no change in colour (no growth).

Results and Discussion

Detailed results of four (4) produced water samples analysed for bacterial growth rate and pattern, using serial dilution method are discussed as follows. The growth rate (culturing) of bacteria (SRB) after 28 days is shown in the appendixes, B and C. Enumeration of the number of bacteria in the samples are taken at the end of the 28th day (this covers the total number of bacteria present in the samples), and these are shown below, through a graphical representation of the overall growth for 1 month (figures 1.1 to 1.3).

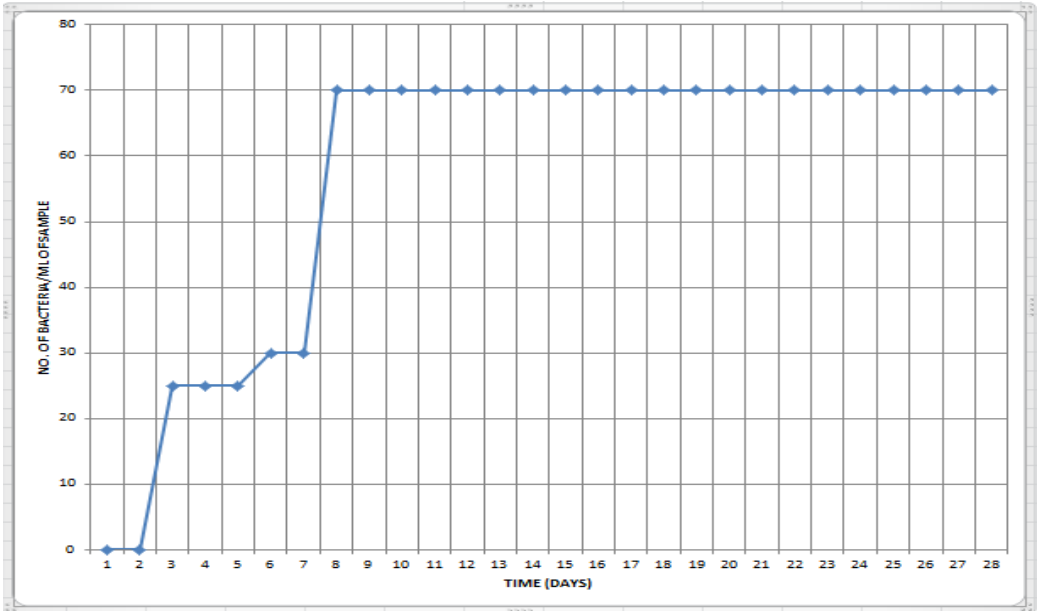


Figure 1.0: Graphical representation of bacteria growth over time for sample 1a.

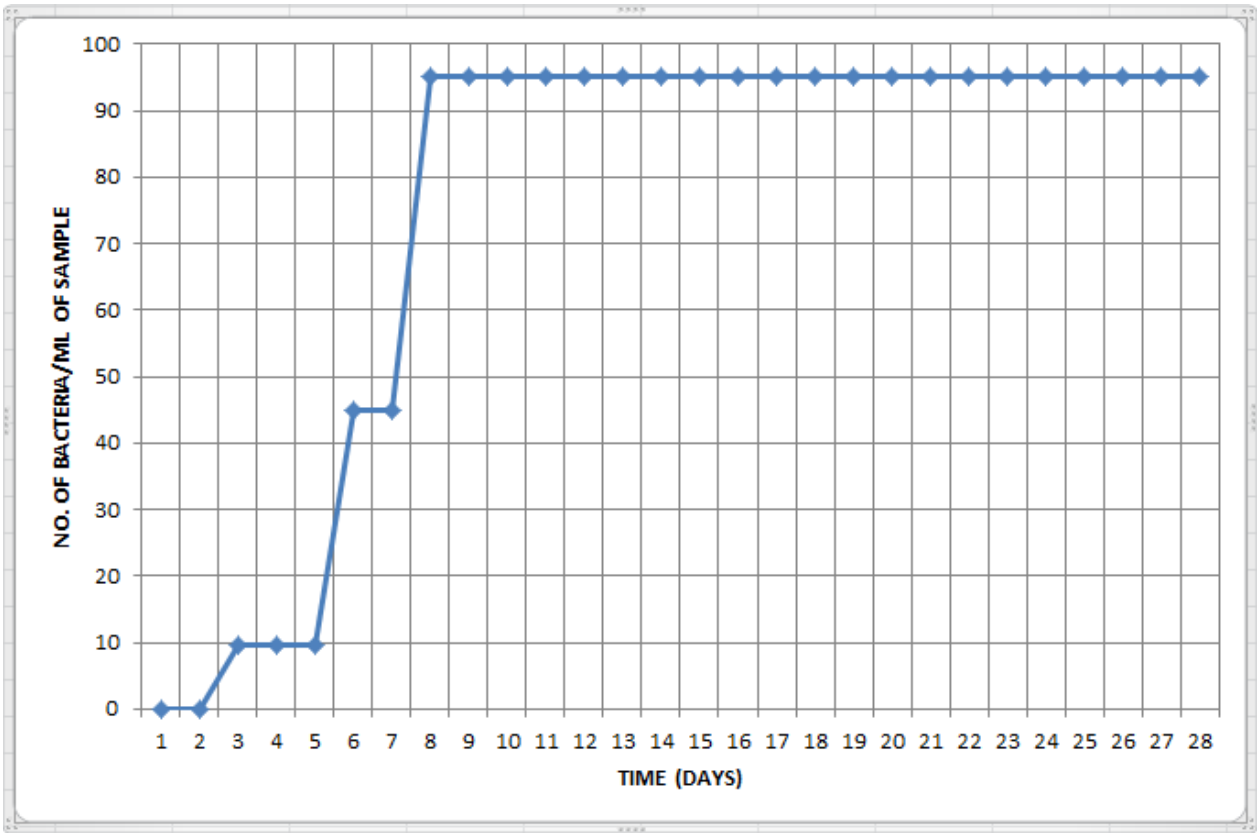


Figure 1.1: Graphical representation of bacteria growth over time for sample

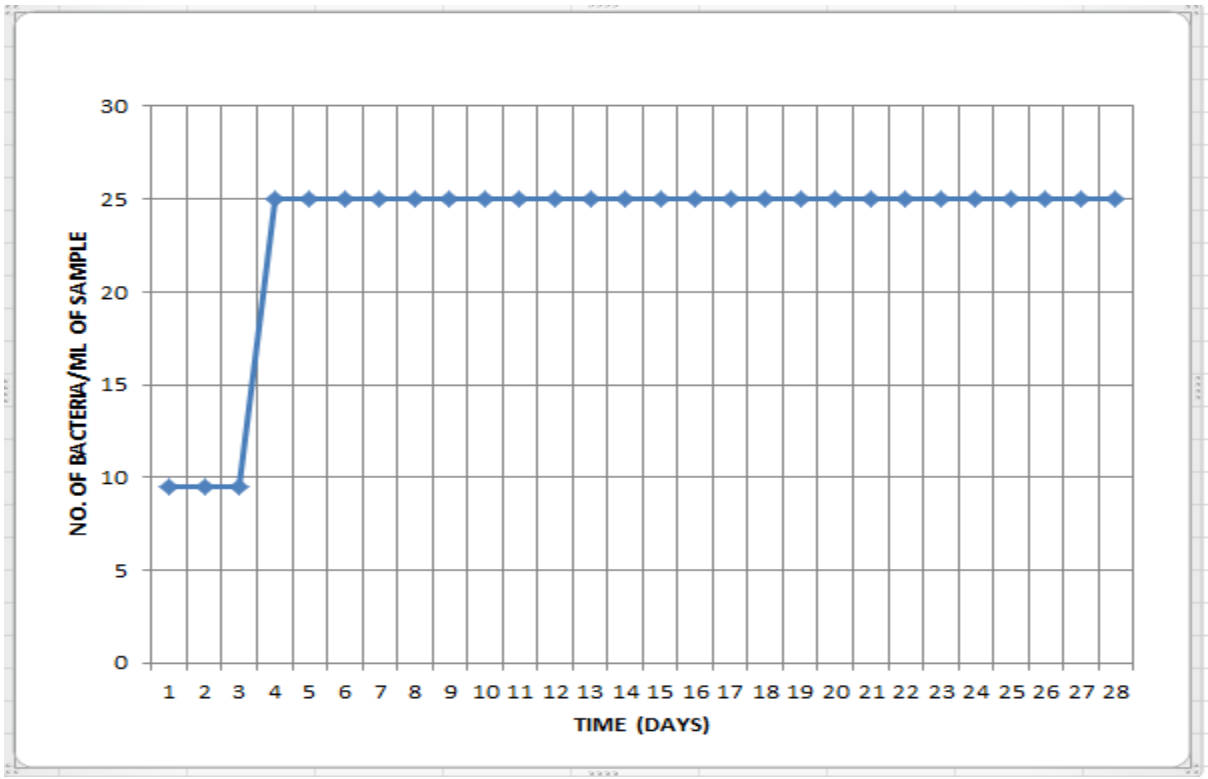


Figure 1.2: Graphical representation of bacteria growth over time for sample 2.

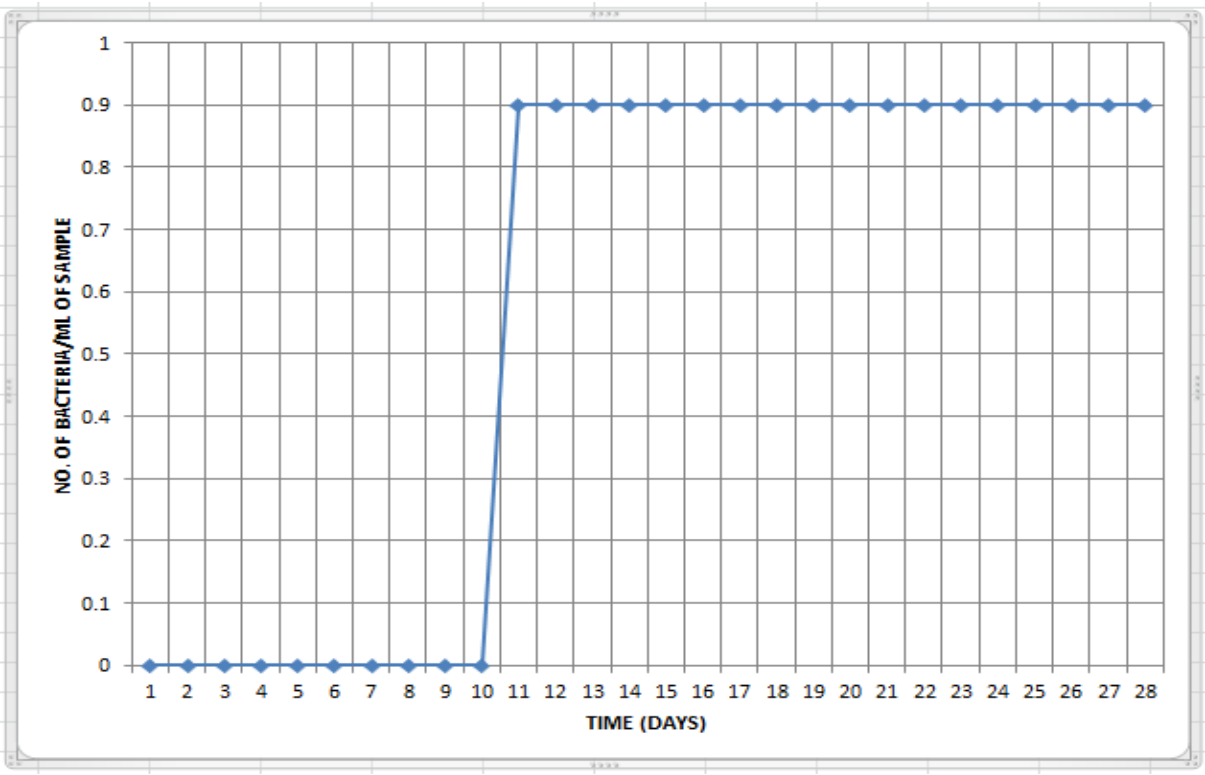


Figure 1.3: Graphical representation of bacteria growth over time for sample 3.

Growth Pattern

Sample 1A:

From appendix A, for sample 1A (Before), there was no growth for the first two (2) days, then the 3rd day growth commenced from 1st vial to the 6th vial and lasted till the 5th day. From 6th to 7th day there was a growth increase only on 12th vial. Also from the 8th day there was an increase in growth in the number of the vial (from the first vial to the 7th, and the 12thvial) which lasted till the 28th day.

Sample 1B:

From appendix B, for sample 1B (After), there was no growth for the first two (2) days, then the 3rd day growth commenced (from the 1st to the 4th vial, then the 6th vial) and lasted till the 5th day. From 6th to 7th day there was a growth increase in the 5th and 7th vial. Also from the 8th day there was an increase in growth in the number of the vial (from the first vial to the 8th) which lasted till the 28th day.

Sample 2:

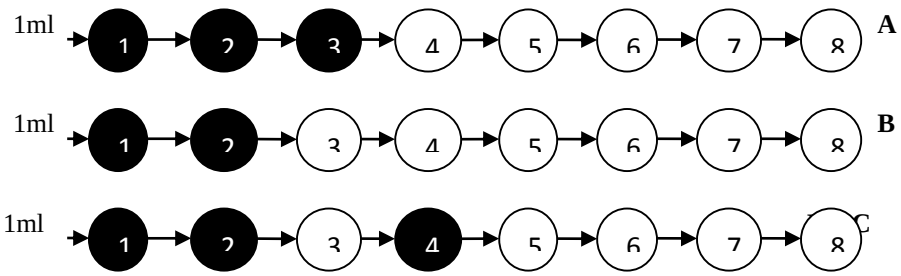
From appendix C, for sample 2, there was growth for the first three (3) days, (from 1st to 3rd vial, then the 5th and 6th vial), no growth was found in 4th vial. Then there was an increase in growth from the 4th day (only in the 4th vial) to the 28th day.

Sample 3:

From appendix C, for sample 3, there was no growth for the first ten (10) days, and then the 11th day growth commenced (from the first 2 vials) and was held constant to the 28th day.

ENUMERATION OF SRB USING MOST PROBABLE NUMBER (MPN) FOR 28 DAYS

Total number of SRB for Sample 1A is taken at the end of 28 days of incubation.

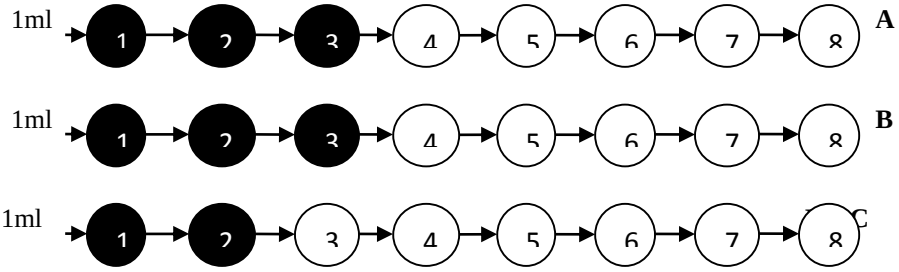


Dil'n of inoculum	10^o	10^1	10^2	10^3	10^4	10^5	10^6	10^7
N	0	1	2	3	4	5	6	7
Pattern		3	3	1	1	0	0	0
x, y, z				x	y	z		

$$x, y, z = 1, 1, 0 \quad this\ pattern\ gives\ 0.7$$

$$x\ dilution = 102\ thus\ result = 102 \times 0.7 = 70\ \boldsymbol{bacteria\ per\ ml\ of\ sample.}$$

Total number of SRB for Sample 1B is taken at the end of 28 days of incubation.

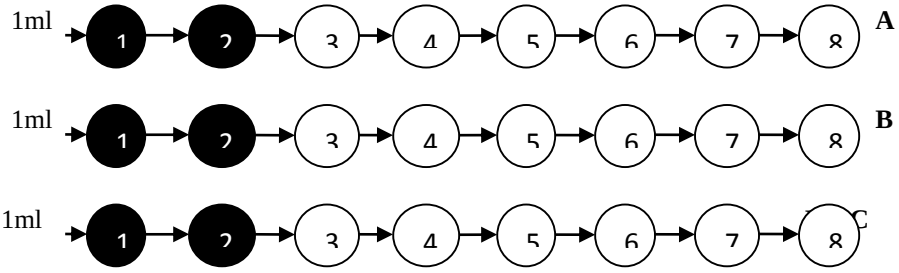


Dil'n of inoculum	10^o	10^1	10^2	10^3	10^4	10^5	10^6	10^7
N	0	1	2	3	4	5	6	7
Pattern		3	3	2	0	0	0	0
x, y, z			x	y	z			

$$x, y, z = 3, 2, 0 \quad this\ pattern\ gives\ 9.5$$

$$x\ dilution = 101\ thus\ result = 101 \times 9.5 = 95\ \boldsymbol{bacteria\ per\ ml\ of\ sample}.$$

Total number of SRB for Sample 2 is taken at the end of 28 days of incubation.

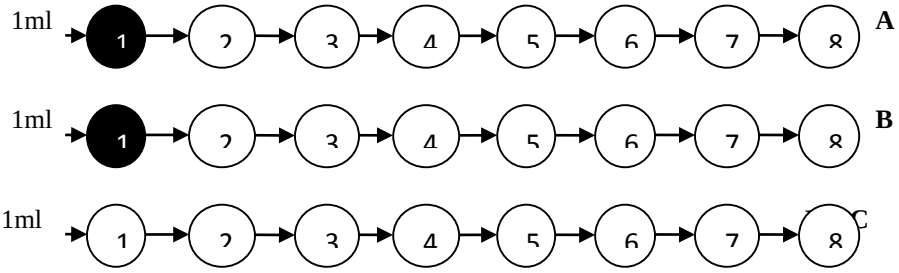


Dil'n of inoculum	10^o	10^1	10^2	10^3	10^4	10^5	10^6	10^7
N	0	1	2	3	4	5	6	7
Pattern		3	3	0	0	0	0	0
x, y, z		x	y	z				

$$x, y, z = 3, 3, 0 \quad this\ pattern\ gives\ 25$$

$$x\ dilution = 100\ thus\ result = 100 \times 25 = 25\ \boldsymbol{bacteria\ per\ ml\ of\ sample}.$$

Total number of SRB for Sample 3 is taken at the end of 28 days of incubation.



Dil'n of inoculum	10^o	10^1	10^2	10^3	10^4	10^5	10^6	10^7
N	0	1	2	3	4	5	6	7
Pattern		2	0	0	0	0	0	0
x, y, z		x	y	z				

$$x, y, z = 2, 0, 0 \quad this\ pattern\ gives\ 0.9$$

$$x\ dilution = 100\ thus\ result = 100 \times 0.9 = 0.9\ \boldsymbol{bacteria\ per\ ml\ of\ sample.}$$

The method used in the interpretation of the bacteria result is applicable for any broth media triplicate. Most Probable Number result performed in 9ml dilutions.

From figure 1.0 below, there was no growth from the first 2 days, then a constant growth rate from 3rd to 5th day, after gaining energy, the growth increased from 6th to 7th day, also from the 8th day the growth remained constant. From figure 1.1, sample 1B has the same growth pattern with sample 1A, but different bacteria number, that is why the growth pattern does not directly tell the number of bacteria but indirectly helps in its enumeration. From figure 1.2, the growth started immediately after inoculation, and there was a constant increase in growth from 4th day. From figure 1.3, the sample experience a late positive (no growth) for ten days, then growth commenced on the 11th day and was held constant.

DISCUSSION

From the results of the growth rate and pattern of the sulphate reducing bacteria in produced water samples carried out. It was observed that a constant growth rate from 8th to 28th day (figure 1.0), (figure 1.1), from 4th to 28th day (figure 1.2) and from 11th to 28th day shows the stationary phase (constant growth). This indicates the exhaustion of essential nutrients, or accumulation of toxic products of metabolism, it normally limits the period of high growth rate. At the end of the exponential growth phase (increase in growth rate), the growth rate and death rate reaches equilibrium. At this point, the bacteria are dormant, and will continue in this state until nutrient (sulphate water) is provided for it to be active.

Also, the early growth that some of the samples experience are due to the high level of H_2S present in the sample before culturing, the sampled water turns black already before sampling in figure 1.2 (in the case of sample 2) due to the high level of H_2S present in the sample, but not as a result of bacteria growth.

For the case of sample 3 in figure 1.3, that experienced late positive growth, it is necessary that vials should be retained for full 28 days to check for late positive, but it may not be necessary keeping the samples for up to 28 days when the sample experiences early positive.

CONCLUSION

The overall economic impact of bacterial challenges as a result of disposing produced water into the sea and their possible impact on production strings in terms of metallic corrosion can be highly damaging, yet most produced water treatment methods does not include consideration for preventing the growth of bacteria. Attempts to prevent the introduction of bacteria into the system can be almost impossible, since most of them exist naturally in the formation, but to curb the rate at which they grow is the only means of handling the challenge. Sulphate reducing bacteria occur in produced water in the Niger Delta region. Most probable number of SRB for all sample analysed were between 25 – 90 which exceeds the acceptable limit of 20 in 100 ml. In addition, suitable growth conditions for SRB is an anaerobic condition, which is a typical produced water characteristic. Having H_2S in produced water often imply the activity and presence of SRB microorganism. This is characterized by the offensive smell and black precipitate of ferrous sulphide when iron minerals are present. Therefore, prior to disposal of produced water into the environment,

routine produced water treatment methods should include disinfection to take care of sulphate reducing bacteria, and not just toxicity reduction alone.

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APPENDIX A:

Daily growth of Sulphate reducing bacteria for samples 1A and 1B

SULPHATE REDUCING BACTERIA MONITORING SHEET

FIELD	Pre H_2O Sample @20"	Post H_2O Sample @20"
SAMPLING POINT	Kole Creek	Kole Creek
DATE OF SAMPLING	01-03-16	01-03-16
DATE OF INOCULATION	02-03-16	02-03-16

DAYS	BEFORE COLONY												AFTER COLONY											
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-
4	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-
5	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-
6	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-
7	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-
8	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
9	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
10	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
11	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
12	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
13	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
14	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
15	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
16	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
17	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
18	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
19	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
20	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
21	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
22	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
23	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
24	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
25	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
26	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
27	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
28	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-

LEGEND +GROWTH -NOGROWTH INCUBATION TEMP =36.6°C (98°F)

APPENDIX B:

Daily growth of Sulphate reducing bacteria for samples 2

SULPHATE REDUCING BACTERIA MONITORING SHEET

FIELD	Rumaitha
SAMPLING POINT	3rd Rumaitha
DATE OF SAMPLING	3/03/16
DATE OF INOCULATION	4/03/16

DAYS	BEFORE COLONY												AFTER COLONY											
	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}	10^{10}	10^{11}	10^{12}	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}	10^{10}	10^{11}	10^{12}
1	+	+	+	−	+	+	−	−	−	−	−	−												
2	+	+	+	−	+	+	−	−	−	−	−	−												
3	+	+	+	−	+	+	−	−	−	−	−	−												
4	+	+	+	+	+	+	−	−	−	−	−	−												
5	+	+	+	+	+	+	−	−	−	−	−	−												
6	+	+	+	+	+	+	−	−	−	−	−	−												
7	+	+	+	+	+	+	−	−	−	−	−	−												
8	+	+	+	+	+	+	−	−	−	−	−	−												
9	+	+	+	+	+	+	−	−	−	−	−	−												
10	+	+	+	+	+	+	−	−	−	−	−	−												
11	+	+	+	+	+	+	−	−	−	−	−	−												
12	+	+	+	+	+	+	−	−	−	−	−	−												
13	+	+	+	+	+	+	−	−	−	−	−	−												
14	+	+	+	+	+	+	−	−	−	−	−	−												
15	+	+	+	+	+	+	−	−	−	−	−	−												
16	+	+	+	+	+	+	−	−	−	−	−	−												
17	+	+	+	+	+	+	−	−	−	−	−	−												
18	+	+	+	+	+	+	−	−	−	−	−	−												
19	+	+	+	+	+	+	−	−	−	−	−	−												
20	+	+	+	+	+	+	−	−	−	−	−	−												
21	+	+	+	+	+	+	−	−	−	−	−	−												
22	+	+	+	+	+	+	−	−	−	−	−	−												
23	+	+	+	+	+	+	−	−	−	−	−	−												
24	+	+	+	+	+	+	−	−	−	−	−	−												
25	+	+	+	+	+	+	−	−	−	−	−	−												
26	+	+	+	+	+	+	−	−	−	−	−	−												
27	+	+	+	+	+	+	−	−	−	−	−	−												
28	+	+	+	+	+	+	−	−	−	−	−	−												

LEGEND +GROWTH -NOGROWTH INCUBATION TEMP =36.6°C (98 °F)

APPENDIX C:

Daily growth of Sulphate reducing bacteria for samples 3

SULPHATE REDUCING BACTERIA MONITORING SHEET

FIELD	NKPOKU
SAMPLING POINT	3rd NKP @ Pre-H_2O Sample
DATE OF SAMPLING	8/8/16
DATE OF INOCULATION	8/8/16

DAYS	BEFORE COLONY												AFTER COLONY											
	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}	10^{10}	10^{11}	10^{12}	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}	10^{10}	10^{11}	10^{12}
1	-	-	-	-	-	-	-	-	-	-	-	-												
2	-	-	-	-	-	-	-	-	-	-	-	-												
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27	+	+	-	-	-	-	-	-	-	-	-	-												
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LEGEND +GROWTH -NOGROWTH INCUBATION TEMP =36.6°C (98 °F)