Inoculation of Sulphate Reducing Bacteria (SRB) in Crude Oil from Oil Producing Wells in Niger Delta, Nigeria

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Abstract: - **Water produced from three crude oil samples obtained from the Niger Delta area of Nigeria were inoculated to determine the presence of Sulphate Reducing Bacteria (SRB). Produced water from samples A, B and C were obtained by spinning the crude samples using a Rotanta Petroleum Centrifuge set at 40 ^{0}C and 1500 rpm. 1 ml of the inoculum (produced water) from each of the crude samples was introduced into six inoculating test bottles containing saline solution of six different concentrations 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} g/mol respectively with the aid of six syringes by serial dilution. The six inoculated test bottles were incubated in an incubator set at a temperature between 37 - 40 ^{0}C for 28 days with each bottle observed each day. The presence of Sulphate Reducing Bacteria (SRB) was confirmed by the appearance of a black residue in each test bottle and the quantity of Sulphate Reducing Bacteria (SRB) in colony forming unit is determined by the concentration of the inoculating medium the bacteria appeared. The lower the concentration of the inoculating medium, the higher the bacteria count in colony forming unit per mol (CFU/mol). Results obtained showed that Sample A has a bacterial count of <10 CFU/mol, Sample B has a bacterial count between 100 – 1000 CFU/mol while Sample C has a bacterial count between 1 – 10 CFU/mol. Inoculation of SRB in crude oil samples is very important in determining the bacterial count in crude samples and this is critical in understanding the concentration and frequency of bactericides required in the treatment of Sulphate Reducing Bacteria.**

Key Words: **- Bactericide, sulphide, inoculation, saline, concentration, temperature.**

I. INTRODUCTION

Sulphate Reducing bacteria (SRB) are a family of problematic anaerobic bacteria basically of *Desulfovibro* genus, they are found in oil field operations and water injection disposal sites. Sulphate Reducing bacteria are anaerobic because they do not require oxygen to survive unlike the aerobic bacteria, they utilize sulphate (SO_4^{2-}) rather than molecular oxygen for respiration thereby reducing it to hydrogen sulphide (H_2S) (Muyzer and Stams, 2008). Aerobic bacteria can be eliminated by drastically reducing the dissolved oxygen concentration of their medium to the barest minimum of < 20 ppb through the use of oxygen scavengers whereas Sulphate reducing anaerobic bacteria can only be eliminated through batch treatment using bactericides at adequate concentration (Barton *et al.,* 2009). Bactericides are acidic solutions used to curb the growth and replication of bacteria, they provide an unfavorable medium for bacteria to survive. Usually two different bactericides with different chemical compositions are recommended for the treatment of SRB with the bactericides alternated on a weekly basis, the essence is to prevent SRB from developing an immunity against the bactericides. The frequency of bactericide treatment depends on the bacterial count in the crude sample (Plugg *et al.,* 2011). SRB can lead to the souring of sweet production systems by converting sulphates to hydrogen sulphide (H_2S) [10]. H_2S is both soluble in oil and aqueous solution and is dangerous to humans at temperatures as low as 1000 ppm. Bacterial agents can produce H_2S as much as 10,000 ppm in produced fluids (Ruckert, 2016). Sulphate reducing bacteria corrode iron compounds by producing highly corrosive product in addition to hydrogen sulphide and this is highly dependent on the time of formation of iron sulphide film with hydrogen sulphide. If the formation of the iron sulphide film occurs before the formation of a highly corrosive substance then corrosion will be inhibited however if the corrosive substance comes in contact with iron before the formation of the iron sulphide film then corrosion is bound to occur. Sulphate reducing bacteria can also lead to the corrosion of lead, copper and other metals (Bontognali *et al.,* 2014). Bacteria can exist in down hole / surface equipment, pumps, tubings and the formations itself. The presence of bacteria can increase corrosion and plugging of filters. Bacteria can also form biofilms which are capable of reducing well injectivity (Liamleam and Annachhatre, 2007). The picture of a typical sulphate reducing bacteria is shown in Figure 1 (Castañeda-Carrión *et al.,* 2010).

Bacterial inoculation can be defined as the introduction of bacteria into a suitable medium where they can be nurtured to ascertain their availability both qualitatively and quantitatively. Qualitatively refers to the particular type of bacteria present while quantitatively refers to the amount or quantity of bacteria present (Bontognali *et al.,* 2008). Due to the microscopic nature of bacteria they are usually not accessed directly rather they are contained in a suspected medium (inoculum), this suspected medium is introduced into the inoculating medium. The aim of this study is to inoculate crude oil samples from Niger Delta, Nigeria to ascertain the

presence of sulphate reducing bacteria (SRB). Inoculation of the crude samples provides knowledge of the quantity of SRB present in the sample and this is critical in knowing the frequency and concentration of bactericide treatment required (Austerjost *et al.,* 2017).

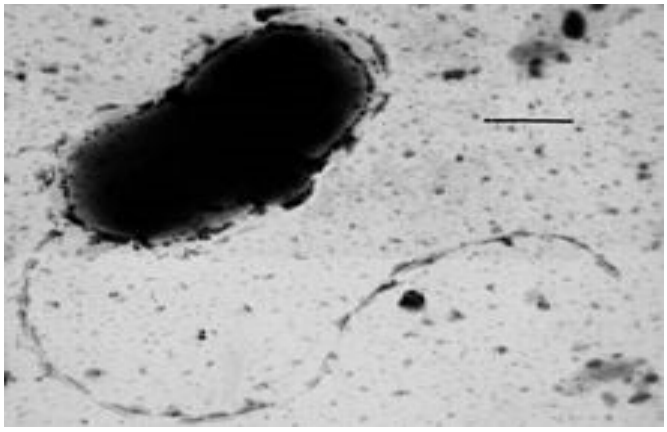

Fig 1 Sulphate Reducing Bacteria (Desulfovibrio vulgaris)

II. MATERIALS AND METHODS

Sample Collection and Preparation

Crude oil samples with a record of high water cut (> 2%) labelled A, B and C were obtained from three (3) different producing wells in the Niger Delta area of Nigeria using glass sampling bottles. The sampling bottles were rinsed properly with xylene, air dried and rinsed with the sample. The entrapped water from the samples obtained with the use of a 460R Rotanta Petroleum Centrifuge were inoculated for Sulphate Reducing Bacteria (SRB).

Determination of Water Cut of Crude Oil

The water cut of each crude sample was determined using Rotanta 460R Petroleum Centrifuge set at 40 ^{0}C and 1500 rpm. The produced water from the crude was obtained with the use of a clean pipette immersed into the crude sample contained in a centrifuge bottle sucking up the water from the base. The water obtained was inoculated for SRB.

Determination of Bacterial Count (SRB) in Crude Oil Samples by Inoculation

Produced water obtained from the crude oil samples were inoculated using a kit containing six SRB test bottles corresponding to the concentration 10^0 to 10^{-5} g/mol which in this case represents the dilution factor. Each test kit bottle contains solutions of sulphate and ferrous ions. With a new 2 ml syringe, 1 ml of produced water from the crude was introduced into SRB test bottle labelled 10^0. Bottle 10^0 was agitated to mix properly. With a new syringe, 1 ml was removed from bottle 10^0 and injected into bottle 10^{-1}. The above procedure was repeated until all six bottles were inoculated by serial dilution using six different syringes. The six inoculated test bottles within the kit were incubated in an incubator set at a temperature as close as possible to that of the *in situ* temperature of the sample (between 37 ^{0}C – 40 ^{0}C with a maximum tolerance of +/- 10^0) for a total of 28 days. Each of these test bottle was examined each of these days. The appearance of a black residue in each bottle indicates the presence of SRB. SRB are measured in colony forming unit (CFU/ml) (Silvio *et al.,* 2012).

The bacteria count of each sample is dependent on the inoculating bottle that formed a black residue as shown below:

10^0 - <10 CFU/ml

10^{-1} - 1 – 10 CFU/ml

10^{-2} - 10 – 100 CFU/ml

10^{-3} - 100 – 1000 CFU/ml

10^{-4} - 1000 – 10000 CFU/ml

10^{-5} - 10000 – 1000000 CFU/ml

$$SRB\ Count\ (CFU/ml) = \frac{Quantity\ of\ Bacterial\ colonies \times Dilution\ Factor}{Volume\ of\ Inoculum} \quad \ldots (1)$$

III. RESULTS AND DISCUSSION

Table-1, Inoculation of SRB in Sample A

Observation							
Inoculation	**Date of inoculation**	**Dilution (Bacterial Count, CFU/ml)**					
		10^0 (<10)	10^{-1} (1-10)	10^{-2} (10-100)	10^{-3} (100-1000)	10^{-4}(1000-10000)	10^{-5} (10000-1000000)
Before batch treatment	**1/8/2019**	8D	NC	NC	NC	NC	NC
Batch Treatment							

D = Day bacteria was formed, NC = No change (No bacteria)

Table-2, Inoculation of SRB in Sample B

Observation							
Inoculation	**Date of inoculation**	**Dilution (Bacterial Count, CFU/ml)**					
		10^{0} (<10)	10^{-1} (1-10)	10^{-2} (10-100)	10^{-3} (100-1000)	10^{-4}(1000-10000)	10^{-5} (10000-1000000)
Before batch treatment	**1/8/2019**	4D	14D	18D	22D	NC	NC
Batch Treatment							

D = Day bacteria was formed, NC = No change (No bacteria

Table-3, Inoculation of SRB in Sample C

Observation							
Inoculation	**Date of inoculation**	**Dilution (Bacterial Count, CFU/ml)**					
		10^{0} (<10)	10^{-1} (1-10)	10^{-2} (10-100)	10^{-3} (100-1000)	10^{-4}(1000-10000)	10^{-5} (10000-1000000)
Before batch treatment	**1/8/2019**	6D	16D	NC	NC	NC	NC
Batch Treatment							

D = Day bacteria was formed, NC = No change (No bacteria)

Table-4, Bacteria (SRB) Count in Crude Oil Samples

Samples	**Bacteria Count (CFU/ml)**	**Recommendation**
A	<10	Bactericide batch treatment not required
B	100-1000	Bactericide batch treatment required
C	1-10	Bactericide batch treatment not required

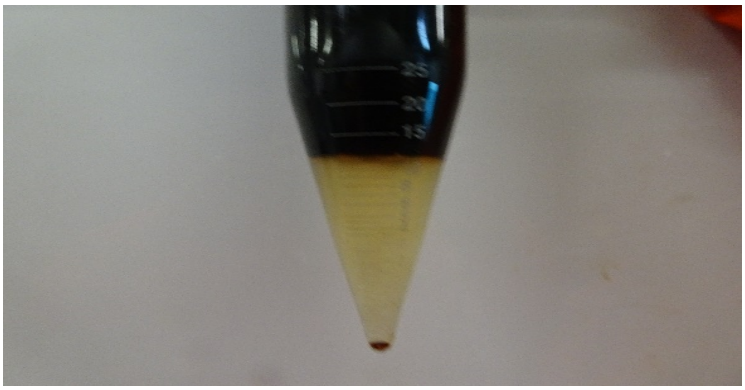

Fig 2: Crude oil Separated into Oil and Water Phase

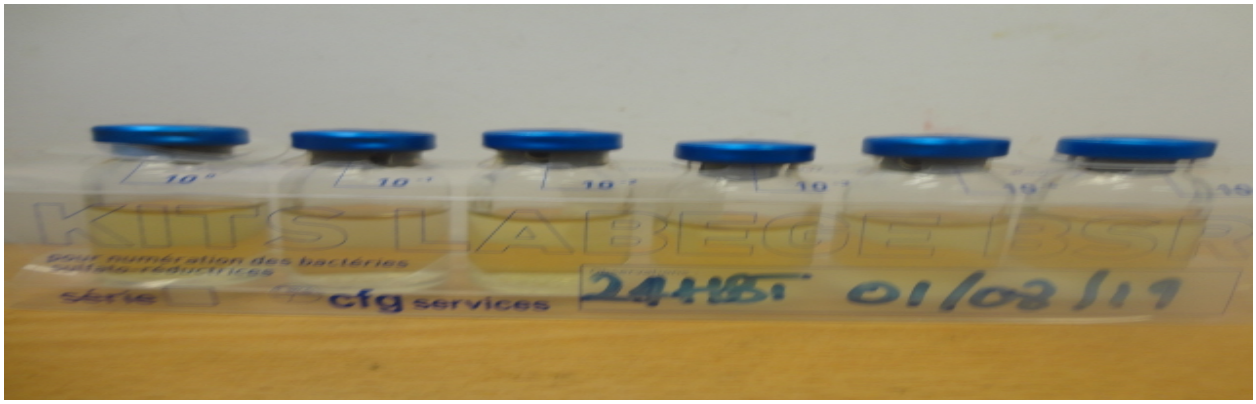

Fig 2: 24 hours before biocide treatment (24HBT) for Sample A (1st Day)

Fig 3: 24 hours before biocide treatment (24HBT) for Sample B (1st Day)

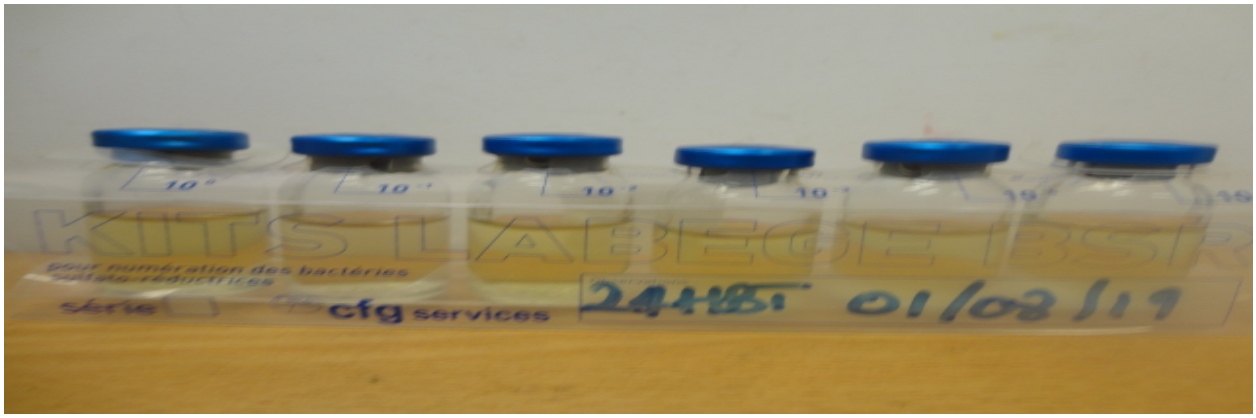

Fig 4: 24 hours before biocide treatment (24HBT) for Sample C (1st Day)

Fig 5: 24 hours before biocide treatment (24HBT) for Sample A (28th Day)

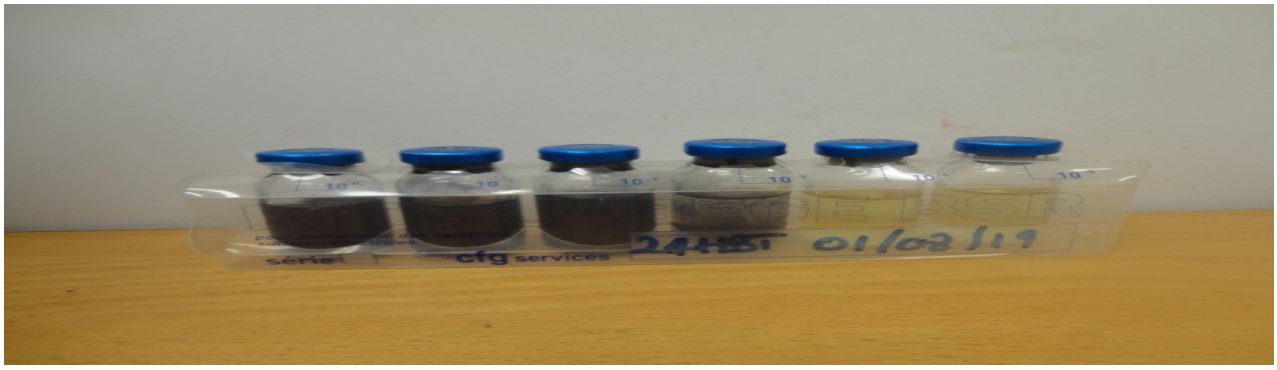

Fig 6: 24 hours before biocide treatment (24HBT) for Sample B (28th Day)

Fig 7: 24 hours before biocide treatment (24HBT) for Sample C (28th Day)

The impact of SRB in oil production facilities has been a cause for concern over the years as such a lot of treatment programs are put in place to curb the prevalence of these bacteria in crude oil and this is important to protect integrity of the crude, the facility and the environment. One very important factor that must be considered in choosing a treatment program for the fight against SRB is an in depth knowledge of the bacteria count in the crude (Bontognali *et al.,* 2014). SRB in crude oil basically reside in the water phase of the crude hence the extraction of the entrapped water from the crude is essential in the study of the bacteria count in crude oil. In fact it is the water phase of the crude that is being inoculated and it is referred to as the inoculum, a typical example of crude oil separated into oil and water phase is shown in Figure 2. Water produced from crude samples (inoculum) suspected to contain SRB were introduced into an inoculating medium contained in a kit by serial dilution. The kit is made up of six glass bottles containing saline solution (inoculating medium) at different concentrations to ascertain the availability of sulphate reducing bacteria in the sample. The concentration of the saline solution at which the SRB grows determines the quantity of SRB within the system and bacteria are quantified in colony forming unit per milliliter. Colony-forming unit can be defined as the unit used in estimating the number of viable bacterial or fungal cells within a medium (inoculum) (Lollar et al., 2019; Austerjost *et al.,* 2017). A sample could contain a lot of dead bacterial cells however colony forming units represents only the number of viable cells in the sample. Viability in this case refers to the ability of the cells to replicate in other words colony forming unit represents the number of bacterial cells in the inoculum that has the ability to replicate. The colony forming unit of an inoculum is calculated using equation 1. The quantity of the bacterial colonies is determined by the dilution factor of the inoculating medium which in this case is saline solution as well as the concentration of the inoculum. The lower the concentration of the saline solution, the higher the bacteria count (Silvio *et al.,* 2012). Bacterial inoculation must be carried out at conditions suitable for bacterial growth and survival, saline solution provides an adequate environment for the survival of SRB. SRB feeds on solutions of sodium chloride (NaCl) and can survive at a reasonable period of time between 14 and 28days within saline solution (Austerjost *et al.,* 2017). Other factors that influences bacterial growth and survival are pH and temperature. SRB are sensitive to mild acidic conditions, they are inactivated at pH values lower than 5, however recent studies have shown that microbial sulphate-reduction can still take place in environments with a pH less than 5 (Ye *et al.,* 2012). Sulphate reduction by microorganisms has been observed in sulphate acidic soils, lakes, wetlands, mesocosms, and bioreactors. It is however worthy to note that the rate of sulphate reduction is reasonably low within an acidic medium. The best pH range for the inoculation of SRB is within an alkaline range of 9 and 10, this is the best pH range at which SRB produces sulphides and it explains the reason why SRB are destroyed with the use of acidic bactericides (Zhalnina *et al.,* 2015). SRB are heterotrophic microorganisms that use low molecular mass organic acids and alcohols as carbon / energy substrates with the organic substrates oxidized either completely to CO2 or to some intermediate compounds such as ethanoic acid using sulphate as a terminal electron acceptor to produce sulphide as shown in equations 2 and 3:

$$Organic\ acids + SO_4^{2-} = CH_3COO^- + HS^- + HCO_3^- \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$Metal + HS^- = Metallic\ Sulphide + H^+ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The most conducive temperature range for bacteria growth is between 40 ^{0}C and 70 ^{0}C, however some bacteria have been known to survive at higher temperatures of 100 ^{0}C. Long term injection of low temperature injection water in high temperature deep formations results in cooler formations around the injector thereby favoring the growth of bacteria (Teske *et al.,* 2002). Figures 2, 3 and 4 shows the inoculum (water from crude samples) in the inoculating medium (six glass bottles different concentration of saline solution) on the first day of inoculation. Inoculation was carried out in an incubator maintained at a temperature of 37 – 40^{0}C. Table 1 shows the result of inoculation for sample A after 28 days, SRB was formed in the first glass bottle with saline concentration 10^0 g/mol on the 8th day after inoculation as

confirmed by the black residue in the bottle as shown in Figure 5. Table 2 shows the result of inoculation for sample B after 28 days, SRB were formed in the bottles with saline concentration 10^0, 10^{-1}, 10^{-2} and 10^{-3} g/mol on the 4th, 14th, 18th and 22nd day respectively after inoculation and this is confirmed by the black residues formed in these bottles as shown in Figure 6. Table 3 shows the result of inoculation for sample C after 28 days, SRB were formed in the bottles with saline concentration 10^0 and 10^{-1} g/mol on the 6th and 16th day respectively after inoculation and this is confirmed by the black residues formed in these bottles as shown in Figure 7. The bacterial count of these samples can be calculated based on these observations with the use of equation 1. Table 4 summarizes the SRB count for each of the samples, Sample A has an SRB count of < 10 CFU/ml which implies that bactericide batch treatment is not required as the number of viable bacteria is negligible and does not have the tendency to replicate. Sample B has an SRB count of between 100 – 1000 CFU/ml, this implies that bactericide is recommended to prevent these SRB from replicating thereby resulting in a lot of damage to the process and environment. Sample C has an SRB count between 1-10 CFU / ml slightly above sample A, its result also implies that bactericide batch treatment is not required. SRB formed at lower concentrations of 10^{-4} and 10^{-5} g/mol implies that bactericide batch treatment is urgently required as a matter of emergency. There are two types of bactericide treatments they are preventive and curative treatment. Preventive treatment is required to prevent the growth of bacteria while curative treatment is required to cure the presence of already existing bacteria, curative treatment is usually preferred for cost optimization (Heggendorn *et al.,* 2015).

IV. CONCLUSION

Bacterial inoculation of crude oil is important in determining the bacteria count in crude and this is critical in understanding the exact concentration of bactericide as well as the frequency of treatment required. Inoculation must take place in an inoculating medium that favors the growth and replication of Sulphate reducing bacteria (SRB), parameters such as salinity, pH and temperature are essential for such purposes. Bacterial inoculation was carried out in an inoculating medium of saline solution with different concentration and bacterial count depends on the concentration at which the SRB grows. Results obtained after inoculating three crude oil samples confirms the presence of SRB in all samples at different colon forming units with bactericide batch treatment required only for sample B.

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