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Anaerobic Microbial Activities of a Nigerian Deep Offshore Oil Production Facility that Uses High Sulfate Sea Water for Injection

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: To evaluate the Anaerobic microbiological activities and souring potential of a Nigerian deep water offshore oil production facility with special emphasis on functional group activities.
Methodology: CSB-K medium was used for the anaerobic microbial activity assay such as the ability to reduce sulfate by sulfate reducing bacteria (SRB), oxidize sulfide and reduce nitrate by the sulfide oxidizing, nitrate reducing bacteria (soNRB) and reduce nitrate by the heterotrophic nitrate reducing bacteria (hNRB) while API-RP-38 and ZPRA-5 broth media were used to quantify the presence of SRBs and acid producing bacteria respectively.
Results: Results on the activities of anaerobic microorganisms indicated that sample 3N1(treated produced water) had the highest concentration of SRBs and Acid producing bacteria (10^5 and 10^7 cells/ml respectively), considerable concentrations of heterotrophic nitrate reducing bacteria and sulphide oxidizing nitrate reducing bacteria were also present in the same sample. The partially treated produced water samples (3N2 and 3N4) showed relatively lower concentrations of SRBs and acid producing bacteria. Expectedly very low microbial activity was recorded in the biocide treated injection water (3N3). Comparatively, the highest microbial activity on sulfide oxidation, nitrate reduction, sulfate reduction and production of hydrogen sulfide was recorded in sample 3N1 while the lowest microbial activity was recorded in sample 3N3.

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Conclusion: Anaerobic microbiological activities of the oil field under investigation indicate that the field have high potential for souring and corrosion due to the availability of sulfate, organic nutrients and SRBs but the operators of the field have taken measures to control souring and bio-corrosion which include periodic biocide treatment and nitrate injection.

Keywords: SRB; soNRB; hNRB; produced water; injection water; anaerobic microbiological activities; functional group activities.

1. INTRODUCTION

Microorganisms are found throughout oil production systems from the reservoir rock itself through pipelines and topside facilities and as a result of this, oil reservoirs may be contaminated with some of these microorganisms especially those known to cause corrosion, souring of oil reservoirs, biofouling and plugging of sediments [1-4]. A group of bacteria known as sulphate reducing bacteria (SRB) are responsible for majority of microbial problems in oil production facilities [5,6]. SRB produces hydrogen sulphide gas (H_2S) as a bi-product of respiration. The H_2S gas produced is volatile and toxic, it sours crude oil and gas making it harder to refine into environmentally friendly high quality fuels hence reducing its value. In addition sulphide concentration even below 1 mg/L in water may lead to high corrosion rates and sulphide stress cracking of susceptible steels [6].

A clear understanding of the anaerobic activities of the three main microbial functional groups that can either positively or negatively impact on oil production is very important in the control of notable microbial problems in the oil and gas industry. Sulfate-reducing bacteria (SRB) for instance can initiate an incomplete oxidation of oil organics to acetate and carbon dioxide or complete oxidation of acetate to carbon dioxide and the reduction of sulfate to sulphide [7-10]. Heterotrophic nitrate reducing bacteria (hNRB) can initiate the incomplete oxidation of oil organics to acetate or carbon dioxide and reduction of nitrate to nitrite and then to either nitrogen or ammonia while nitrate reducing, sulphide oxidizing bacteria (NR-SOB) oxidizes sulfide to sulfate or sulfure with nitrate being reduced to nitrite and then to either nitrogen (with NO and N_2O) as intermediates or ammonia without intermediates [10,11]. The implication of this is that while SRB is problem causing, hNRB and so-NRB are beneficial to the environment.

Nitrite which is a product of nitrate reduction by hNRB and the so-NRB is a powerful SRB inhibitor and nitrite have worked efficiently with some biocides to inhibit SRB [10,11]. Nitrate can also inhibit SRB activities by stimulation of hNRB (competitive exclusion). Recently, it has been discovered that so-NRB can be used to control souring by its ability to oxidize sulfide(lowering sulfide levels) and reduce nitrate to nitrite which further inhibit SRB [10,11].

An investigation was conducted on the anaerobic microbial activities of a Nigerian deep offshore oil production facility in Nigeria that uses high sulfate sea water for injection. The oil reservoir is capable of producing about 180,000-200,000 barrels of crude per day over the 20 year life of the field and to maintain the down hole pressure, about 300,000 barrels of sea water is injected daily at a pressure of 200 bars [12-14]. It is expected that the injected sea water with high sulfate concentration can induce souring down hole when the SRBs starts to utilize sulfate and produce hydrogen sulfide.

Steps have been taken by the operators of the oil field in the past to mitigate the formation of hydrogen sulfide and this include the continuous chlorine treatment of the reservoir and injection water and the twice weekly biocide treatment. Most recently, the operator introduced the injection of 45-100 ppm of calcium nitrate downstream to encourage preferential growth of nitrate reducing bacteria which grows rapidly to outcompete the SRB in the utilization of soluble and volatile organics such as lactate and VFA in the reservoir [14]. This treatment protocol has been successful so far but for it to be more effective and economical, its application has to be based on the scientific findings and directions on the nature, diversity and population of the indigenous anaerobic microbial communities of the reservoir and the injection water systems such as the Sulfate reducing bacteria (SRB), Heterotrophic nitrate reducing bacteria (hNRB) and the Sulfide oxidizing, nitrate reducing bacteria (so-NRB) and the environmental conditions that enables them to proliferate such as the availability of nitrate, lactate, VFA and sulfate.

The main objective of the present investigation therefore is to determine the anaerobic functional group activities of the oil field microorganisms present in produced and injection water samples such as the ability to reduce sulfate and generate sulfide by the SRB, reduce nitrate by the hNRB and oxidize sulfide and reduce nitrate by the so-NRB and also the environmental conditions and organic nutrients that facilitates souring and corrosion.

2. MATERIALS AND METHODS

2.1 Sample Collection and Shipment

Samples 3N1 (Treated produced water), 3N2 (Partially treated produced water-1), 3N3 (Treated injection water), 3N4 (Partially treated produced water-2) were collected from Bonga deep water oil field in sterile 500 ml Nalgene sample bottles which were filled to the brim to exclude air. The samples were later shipped to the Petroleum microbiology research laboratory, University of Calgary for analysis.

2.2 Chemical Analysis

The samples were analyzed for pH, SO_4^{2-}, HS^-, NH_4^+, NO_3^-, NO_2^- and organic acids such as acetate, propionate and butyrate. The pH was analyzed using Orion pH meter. SO_4^{2-} was analyzed in two ways, through High Performance Liquid Chromatography (HPLC) and through turbidimetry using $BaCl_2$ [15]. HS^-, a dissolved sulphide was analysed using diamine method [16]. NH_4^+ was analyzed using the indol-phenol method. NO_3^-, NO_2^- and organic acids such as acetate, propionate and butyrate were analyzed using HPLC. SO_4^{2-}, NO_3^- and NO_2^- were analyzed using 100 µL of the samples with 400 µL HPLC anion buffer while organic acid analysis used 300 µL of the samples and 20 µL1 M phosphoric acid.

2.3 Microbiological Assay

The medium that was used for the microbiological assay was Coleville synthetic brine (CSB-K) with composition (g/L) as previously described [17]; NaCl(1.50), $CaCl_2$ $2H_2O$ (0.21), $MgCl_2$ $5H_2O$ (0.54), NH_4Cl (0.30), KCl (0.10), KH_2PO_4 (0.05) Resazurin, (1%)2-3 drops. These chemicals were mixed and dissolved in MQ water in an Erlenmeyer flask and were transferred to a Widdel flask for autoclaving. After autoclaving, more components were added: Trace elements (1 ml), Selenate-tungstate (1 ml), $NaHCO_3$ (1 M) 30 ml, Na_2S (1 M) 1 ml, HCl (2 M) 2 ml, pH adjusted to 7.4. The Widdel flask was connected to a gas stream of 90% N and 10% CO_2. About 70 ml of the medium was then aseptically and anaerobically dispensed to 125 ml serum bottles with a gas phase of 90% N and 10% CO_2 and closed with a sterile butyl rubber stopper.

2.4 Components Added to CSB-K for Specific Microbiological Tests

The following electron donors and acceptors were added to the CSB-K medium in serum bottles to determine the functional group activity of major bacterial groups:

a. Sulfate-reducing bacteria (SRB) – 40 mM lactate and 20 mMsulfate; 3 mM VFA and 20 mM sulphate
b. Heterotrophic nitrate reducing bacteria (hNRB) – 3 mM VFA and 10 mM nitrate
c. Sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) – 5 mMsulfide and 10 mM nitrate

3.5 ml of the samples (5%) were added to the prepared media bottles and incubated at 37°C in a shaker for about 30 days. Using a sterile syringe needle, 1 ml of the sample was taken periodically for every 2 days within the first one week and subsequently for every 7 days and analyzed for sulfide, sulfate, nitrate and nitrite using HPLC. Microbial activities were calculated as $100/t_{1/2}$, where $t_{1/2}$ is the time (days) needed to reduce half of the sulfate (SRB activity), nitrate (hNRB and so-NRB activities) and sulfide concentrations (so-NRB).

2.5 Most Probable Number (MPN) Measurement

To quantify presence of SRB in the samples, API RP-38 broth media were used. Serial dilution of the samples in API RP-38 broth media of up to 10 fold was made. With the use of a sterile syringe needle, 1 ml of each sample was inoculated serially to the 9 ml medium up to the 10^{th} tube making a ten-fold dilution. Samples were then incubated at 37°C for up to 30 days. Formation of black precipitates of iron sulfide was used as a diagnostic tool to confirm the presence of SRB. For acid producing bacteria, prepared ZPRA-5 acid produced media (Phenol red-dextrose reagent) with a salinity of 5000 ppm was used. Change in colour from orange to

yellow shows presence of acid producers (Fermentation of Dextrose).

3. RESULTS

3.1 Physico-chemical Characteristics of Samples

The major organic nutrient in all samples is acetate with concentrations that ranged between 0.07 and 3.20 mM. Propionate was also present at relatively low concentrations in all samples except 3N3 while Butyrate was completely undetected in all samples. As expected both sea water and produced water recorded relatively high sulphate concentrations (10.36-21.69 mM). Amonia was present at considerable considerations in all samples while sulphide and nitrite were below detectable limits in all samples. Detailed results are shown in Table 1.

3.2 Most Probable Number (MPN) Results of Anaerobic Bacteria in Samples after 3 Weeks of Incubation

Both SRB and APB were found at relatively high concentrations in sample 3N1(Treated produced water) which also recorded significant presence of hNRB and so-NRB. As expected, the biocide treated injection water (3N1) recorded lower concentrations of SRB and APB (10^1-10^3 cells/ml) with significant presence of hNRB probably as a result of nitrate injection. So-NRB was present in all samples except 3N3 (Injection water). Detailed results are shown in Plates 1, 2 and Table 2.

3.3 Comparative Analysis of Experimental Samples on the Activities of soNRB, SRB and hNRB

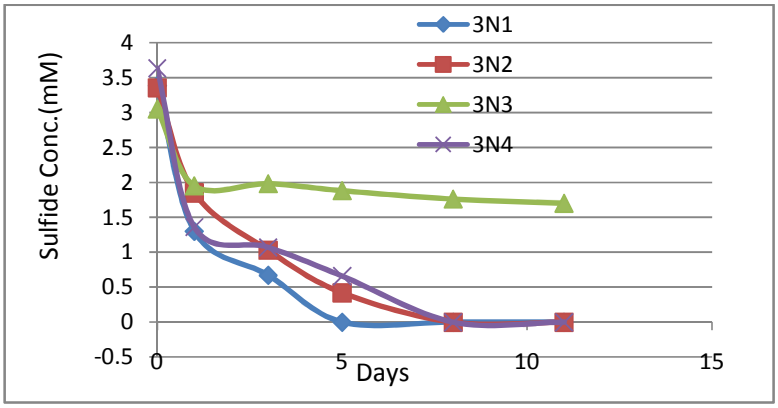
Nitrate was significantly reduced and sulphide was also oxidized considerably by the soNRBs in samples 3N1, 3N2 and 3N4 but no soNRB activity was observed in sample 3N3 as shown in Fig. 1. Sulfate reduction and subsequent production of hydrogen sulphide was significantly low in sample 3N3 (Biocide treated injection water). This is in agreement with the MPN results that recorded very low concentrations of SRB. The other 3 samples (3N1, 3N2 and 3N4) recorded significant reduction of sulphate and subsequent production of hydrogen sulphide as shown in Fig. 2. However, the rate of sulphate reduction and generation of hydrogen sulphide was higher in lactate media than VFA media. Contrary to what was observed with soNRB and SRB, all the samples including 3N3 showed considerable nitrate reduction, an indication that the hNRBs were present in all the samples. Detailed results are shown in Fig. 3.

Table 1. Physico-chemical characteristics of samples

S/N	Sample code	pH	HS^- (mM)	SO_4^{2-} (mM)	NH_4^{2-} (mM)	NO_3 (mM)	NO_2 (mM)	Acetate (mM)	Propionate (mM)	Butyrate (mM)
1	3N1	7.2	0	10.36	0.513	0.04	0	3.20	1.30	0
2	3N2	7.3	0	11.69	0.622	0.06	0	2.16	0.25	0
3	3N3	6.3	0	21.69	0.112	0	0	0.07	0	0
4	3N4	7.1	0	11.81	0.598	0.01	0	2.10	0.16	0

Table 2. Most probable number (MPN) results of anaerobic bacteria in samples after 3 weeks of incubation

S/N	Sample code	SRB/ml	APB/ml	Presence of hNRB	Presence of SO-NRB
1	3N1	10^5	10^7	+	+
2	3N2	10^2	10^5	+	+
3	3N3	10^1	10^3	+	-
4	3N4	10^2	10^4	+	+



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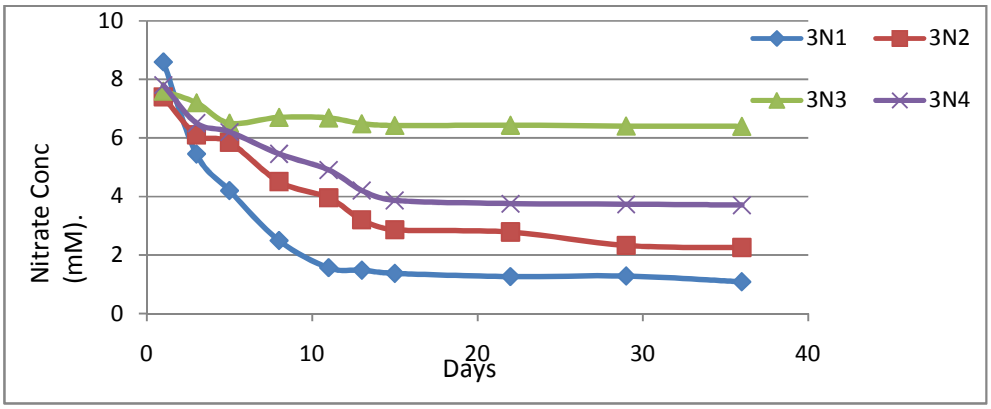

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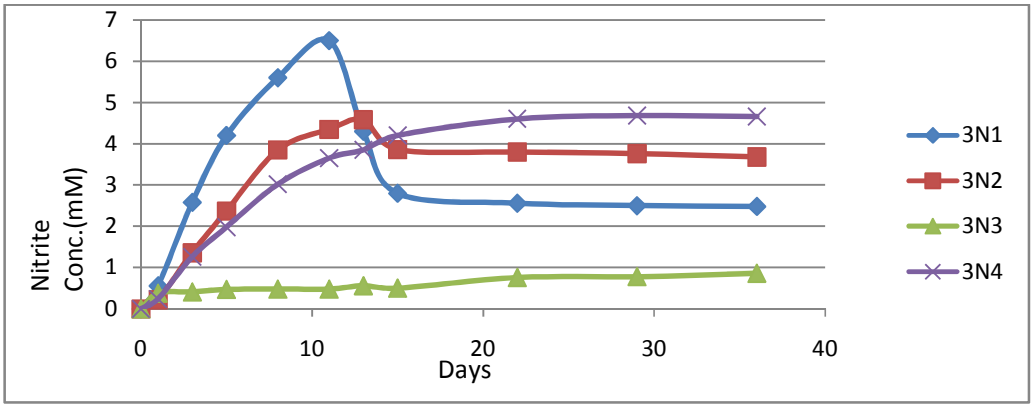

C

Fig. 1. Comparative analysis of experimental samples on the activities of soNRB showing A. sulfide concentration, B. nitrate concentration and C. nitrite concentration

3.4 Anaerobic Microbiological Activity in Sample 3N1 (Treated Produced Water)

There was a considerable reduction of nitrate and oxidation of sulphide by the so-NRBs in sample 3N1. Sulfate was drastically reduced by the SRBs at a much faster rate in lactate media than in VFA media. Same trend was applicable to the rate of generation of hydrogen sulphide. Nitrate was also considerably reduced by the hNRBs as shown in Fig. 4.

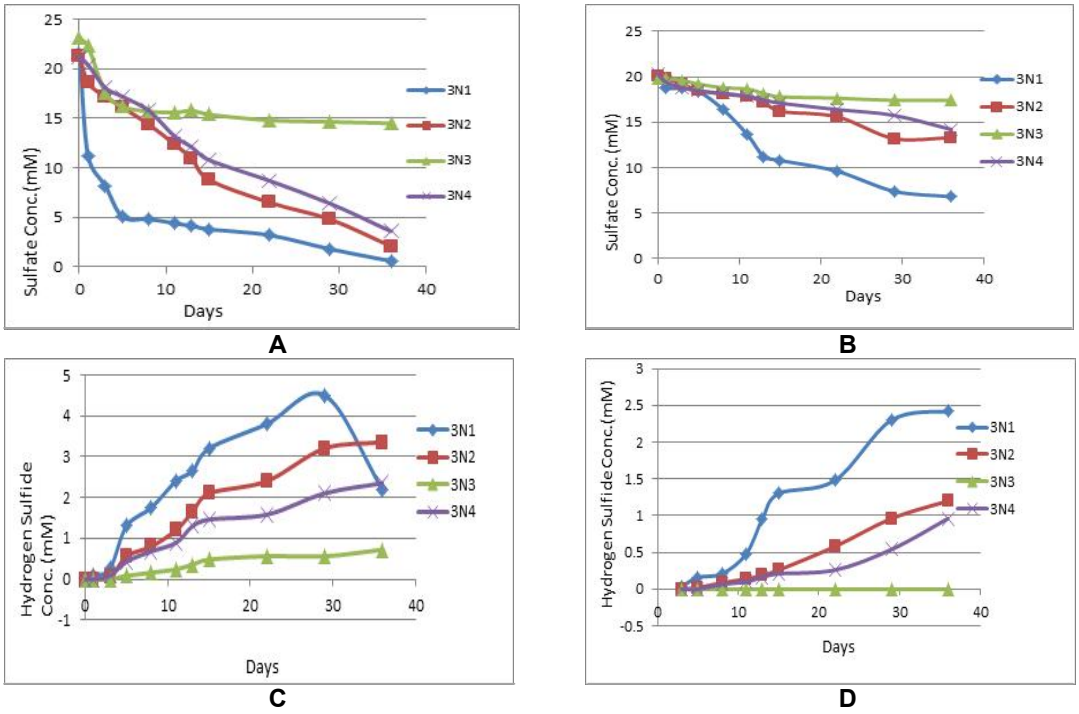

Fig. 2. Comparative analysis of samples on the activities of SRB showing; A. sulfate concentration on lactate media, B. sulphate concentration on VFA media, C. production of hydrogen sulphide on lactate media, D. production of hydrogen sulphide on VFA media

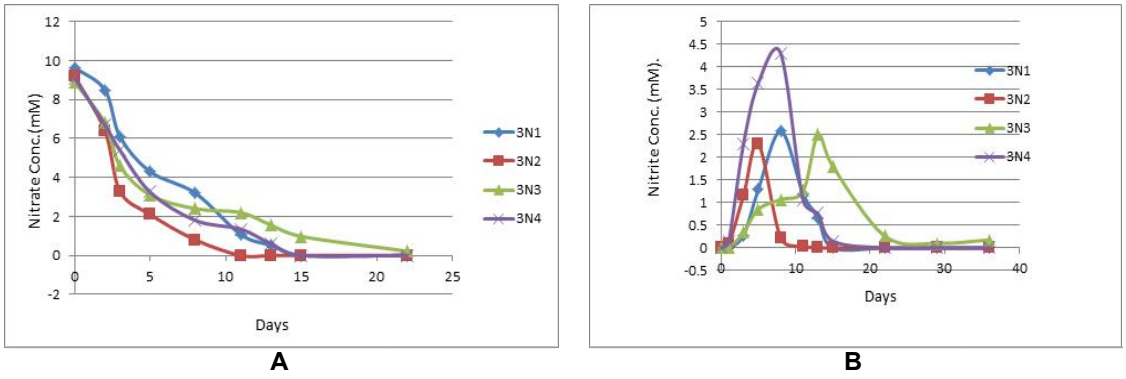

Fig. 3. Comparative analysis of samples on the activities of hNRB showing; A. nitrate concentration, B. nitrite concentration

3.5 Anaerobic Microbiological Activity in Sample 3N2 (Partially Treated Produced Water-1)

The soNRB activities were relatively high in sample 3N2 with drastic oxidation of 3 mM sulphide and considerable nitrate reduction within 10 days of exposure. More than 50% of sulphate was reduced within 2 weeks of exposure with significant generation of hydrogen sulphide. Lactate media was however more preferred than the VFA media. The hNRBs were also very active in sample 3N2 as 100% of original nitrate concentration was reduced within 10 days of exposure. Detailed results are shown in Fig. 5.

3.6 Anaerobic Microbiological Activity in Sample 3N3 (Biocide Treated Injection Sea Water)

Sulfide oxidation and nitrate reduction rates in sample 3N3 were relatively low, confirming the near absence of soNRB in biocide treated injection water sample. Same scenario was applicable to sulphate reduction by the SRBs. Nitrate was however reduced significantly by the hNRB as shown in Fig. 6.

3.7 Anaerobic Microbiological Activity in Sample 3N4 (Partially Treated Produced Water-2)

About 3.5 mM of sulphide was completely oxidized in less than 10 days and nitrate was also considerably reduced by the soNRB. Sulfate reduction rate and subsequent generation of hydrogen sulphide by the SRB was higher in lactate than in VFA media. Detailed results are shown in Fig. 7.

4. DISCUSSION

Sulfide oxidizing, nitrate reducing bacteria (soNRB) were present in sample 3N1 and were very active in the oxidation of sulfide within a relatively short time (5 days). Nitrate was also considerably reduced to nitrite within 15 days. The soNRBs were also present and active in sample 3N2 with a near complete oxidation of sulfide within a period of 8 days and considerable reduction of nitrate to nitrite within 15 days. Same scenario was applicable to sample 3N4, in contrast, sample 3N3 showed relatively low activity of soNRB probably due to biocide action or limited distribution of soNRB species in oil fields [18,19].

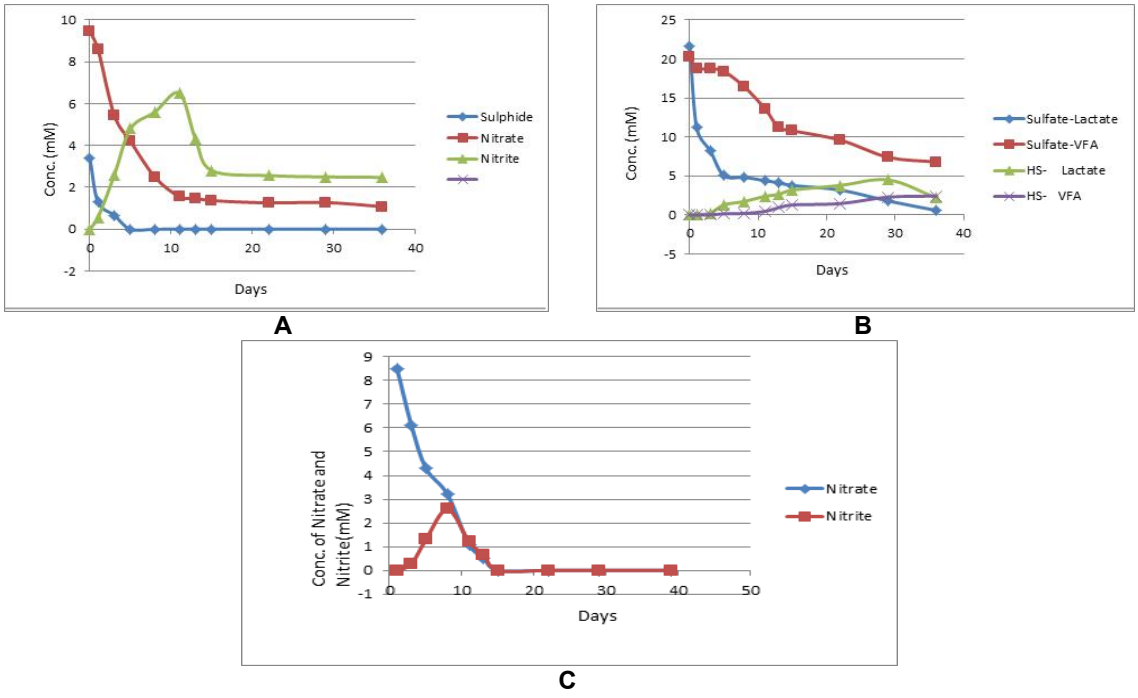

Fig.4. Summary of microbiological assay on sample 3N1, indicating;A. microbiological activities of soNRB showing sulphide, nitrate and nitrite concentrations, B. microbiological activities SRB showing sulphate and sulphide concentrations in both lactate and VFA media, C. microbiological activities of hNRB showing nitrate and nitrite concentrations

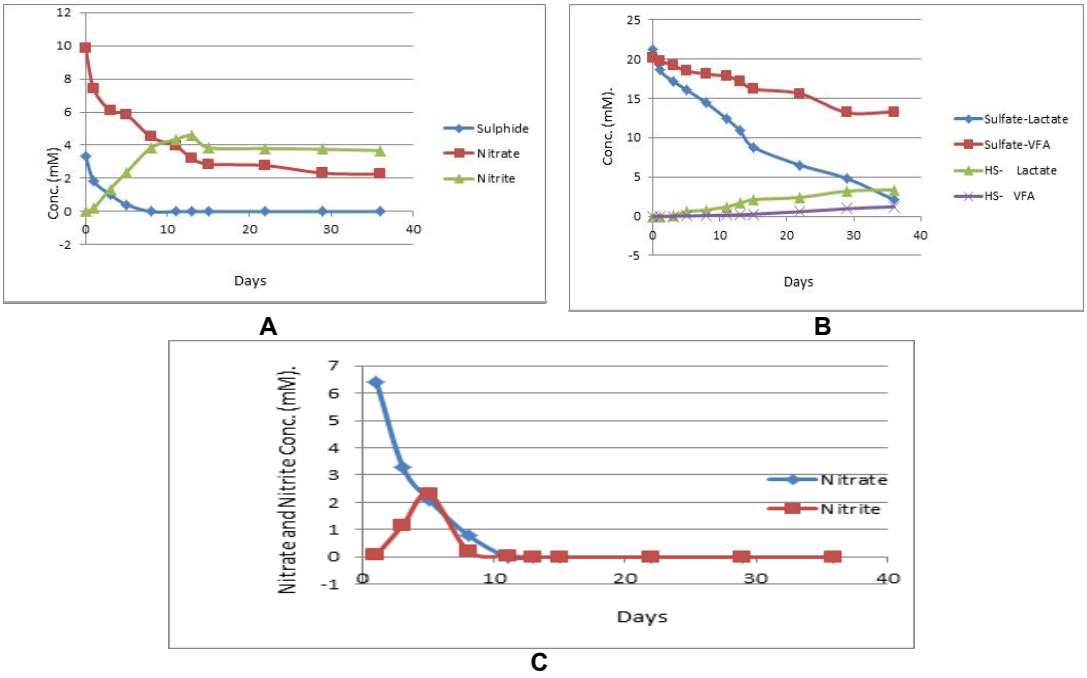

Fig. 5. Summary of microbiological assay on sample 3N2 indicating;A. microbiological activitiesof soNRB showing sulphide, nitrate and nitrite Concentrations, B. microbiological activities of SRB showing sulphate and sulphide concentrations in both lactate and VFA media, C. microbiological activities of hNRB showing nitrate and nitrite concentrations

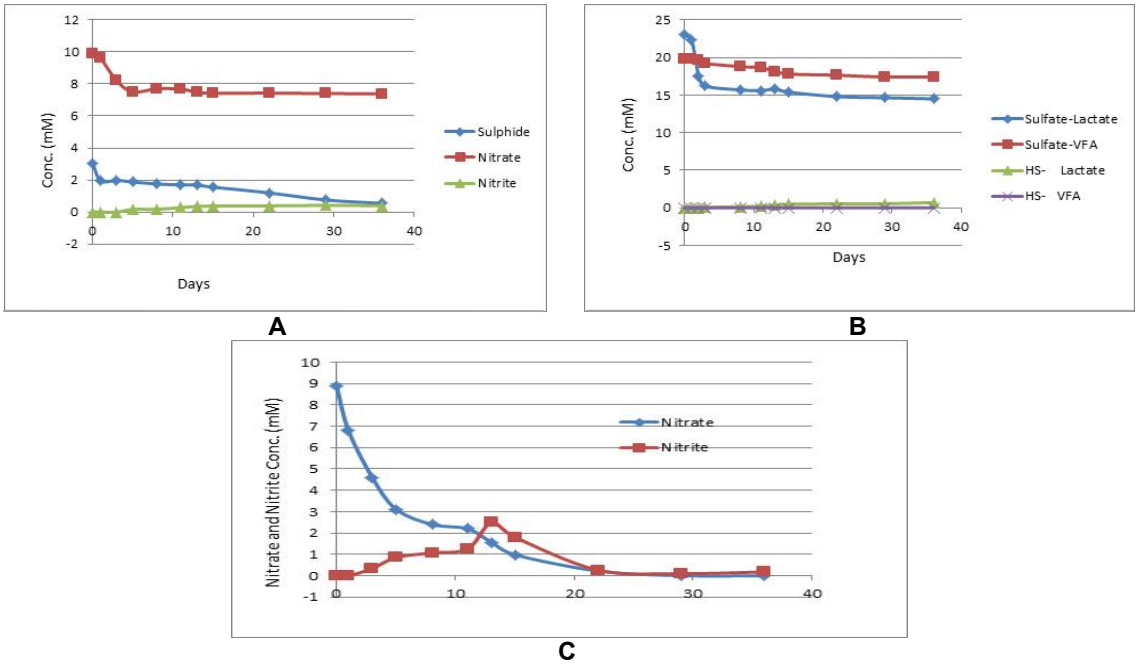

Fig. 6. Summary of microbiological assay on sample 3N3 indicating;A. microbiological activities of soNRB showing sulphide, nitrate and nitrite concentrations, B. microbiological activities of SRB showing sulphate and sulphide concentrations in both lactate and VFA media, C. microbiological activities of hNRB showing nitrate and nitrite concentrations

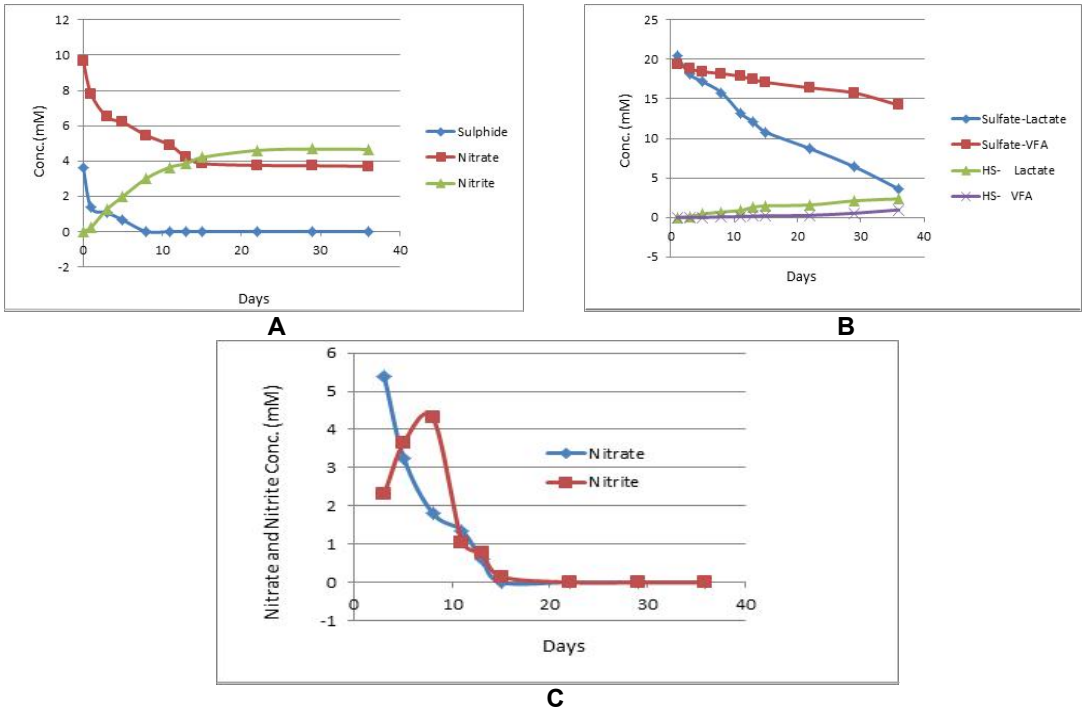

Fig. 7. Summary of microbiological assay on sample 3N4, indicating;A. microbiological activities of so-NRB showing sulphide, nitrate and nitrite concentrations, B. microbiological activities SRB showing sulphate and sulphide concentrations in both lactate and VFA media, C. microbiological activities of hNRB showing nitrate and nitrite concentrations

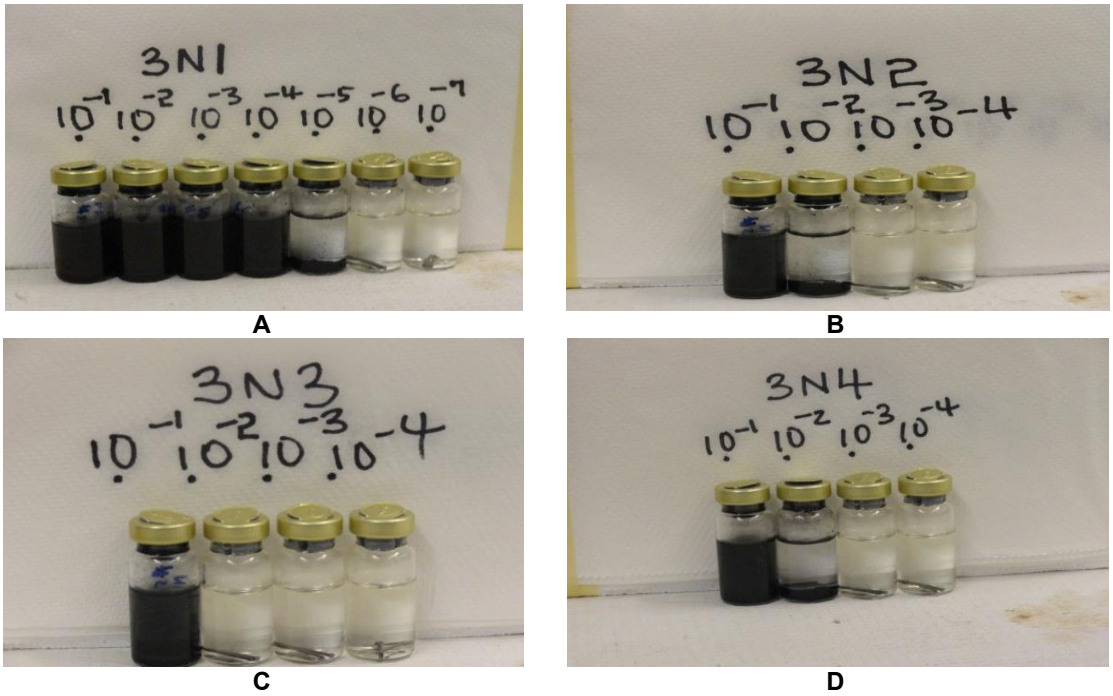

Plate 1. MPN analysis of SRB in samples 3N1(A), 3N2(B), 3N3(C) and 3N4(D)

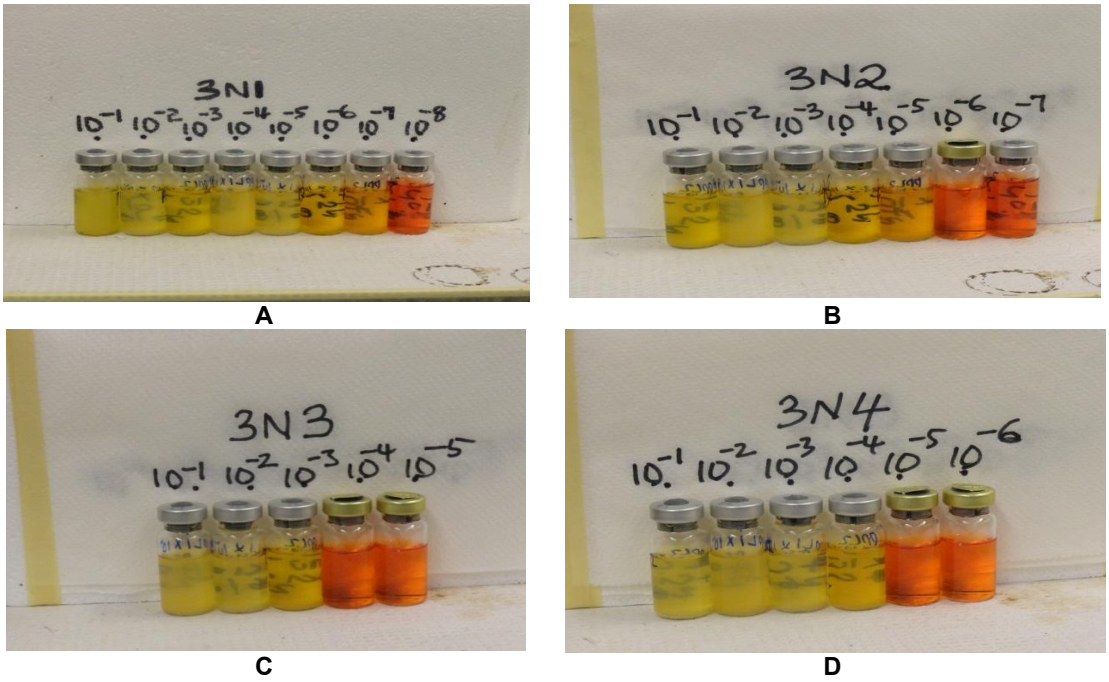

Plate 2. MPN analysis of APB in samples 3N1(A), 3N2(B), 3N3(C) and 3N4(D)

Sulphate reducing bacteria (SRB) were observed to be present in sample 3N1 at relatively high concentration (10^5 cells/ml) and sulphate was reduced considerably in both lactate and VFA media with a corresponding rise in the production of hydrogen sulphide. In consistence with previous investigations, the activity of SRB was higher in lactate than in VFA media [17]. SRBs were also very active in samples 3N2 and 3N4 but sulphate was far more reduced in lactate than in VFA media, same trend followed the production of hydrogen sulfide which was also higher in lactate than in VFA media. Sample 3N3 showed very low SRB activity. This may be as the result of the biocide treatment [14].

The heterotrophic nitrate reducing bacteria (hNRB) showed considerable activity in all the samples including the biocide treated injection water. Some authors have advanced that heterotrophic nitrate reducing bacteria are widely distributed in oil fields [11,18-20] and periodic nitrate injection to this particular field may have contributed to the activation of indigenous nitrate reducers [8,18,21,22].

From our investigation, it can be advanced that the treated produced water sample 3N1 showed the highest microbiological activity as it relates to the activities of the SRB, soNRBs and the hNRB while the biocide treated injection water showed the lowest microbiological activity of the SRBs and the soNRBs but the hNRB showed considerable activity. The partially treated produced water 3N2 and 3N4 collected from different outlets showed similar microbiological activity as it relates to the activities of the SRBs, soNRBs and the hNRB. The three samples that recorded significant microbiological activity (3N1, 3N2 and 3N4 were also richer in organic nutrients (acetate and propionate) than sample 3N3 that recorded lower microbiological activity. There seem to be a strong correlation between the concentration of the SRBs and the APBs in all the samples [23].

5. CONCLUSION

Anaerobic microbiological activities of the oil field under investigation indicate that the field have high potential for souring and corrosion due to the availability of sulfate, organic nutrients and SRBs but the operators of the field have taken measures to control souring and bio-corrosion which include periodic biocide treatment and nitrate injection.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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