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The level of Inhibition of Microbial Functional Group Activities by Some Oxidizing Agents Commonly used as Biocides in Oil field Operations

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Author's contribution

This whole work was carried out by author OCC.

Original Research Article

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ABSTRACT

Aim: To determine the level of inhibition of microbial functional group activities such as the ability to reduce sulfate to sulfide by sulfate reducing bacteria (SRB), reduce nitrate to nitrite by the heterotrophic nitrate reducing bacteria (hNRB), and oxidize sulfide and reduce nitrate by sulfide oxidizing, nitrate reducing bacteria (so-NRB) by some oxidizing biocides like chlorine, bromine and ozone.
Methodology: Samples of the oxidizing biocides were obtained from Microcheck and the inhibition of some functional group activities in produced and injection water samples were determined using CSB-K medium.
Results: Ozone was found to be more effective than chlorine and bromine in the inhibition of functional group activities at lower concentrations.
Conclusion: More research effort is required to see if ozone can work in synergy with other biocides to improve on its efficiency.

Keywords: Functional group; sulfate reducing bacteria; heterotrophic nitrate reducing bacteria; sulfide oxidizing nitrate reducing bacteria; oxidizing biocides; produced water; injection water.

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1. INTRODUCTION

Uncontrolled microbial growth in oil field water systems can lead to expensive problems such as fouling, reservoir plugging, bio-corrosion and souring [1,2]. These problems are conventionally controlled using inorganic or organic chemicals known as biocides. Batch treatments using conventional biocides are inefficient because most bacteria are present on surfaces in protective biofilms while some are resistant to the biocides [2].

Biocides are chemical compounds used to disinfect, decontaminate and sterilize materials (surfaces or objects) in order to eliminate microbiological degradation processes. The mode of action of biocides is to stop the current metabolic activity of the microorganisms causing changes in the proper functioning of cells and consequently death of the microorganisms [3]. Sadip et al., [4] observed that the effectiveness of the biocide depends on several factors which include the concentration, duration of contact, water quality variables, temperature, pH, turbidity, organic matter and dissolved solids.

Oxidizing biocides have been widely used in the petroleum industry in the early 70s and late 90s because of their effectiveness, moderate cost, easy treatability and environmental friendliness [5] but in recent times, there has been reported cases of microbial resistance to these group of biocides [6,7]. Some oxidizing biocides such as Chlorine and Bromine form hypochlorous acid (HOCl) and Hypobromous acid (HOBr) which act as the active agents when added to water. Others such as Hydrogen peroxide and Ozone do not rely on the acid as the active agent.

Chlorine is a widely used biocide but it has limited activity in the presence of organic matter. Bromine provides a wider germicidal activity but corrosive while ozone works faster and not totally consumed as the residual ozone naturally degrades to oxygen leaving no toxic end product [8]. All Oxidizing biocides including Ozone, Chlorine and Bromine kill bacteria by diffusion through the cell wall and then oxidize the enzymes within the cell. Ozone's strong oxidation potential makes it attractive for use as a biocide, but when used in systems with considerable chemical oxygen demand (COD), it is not usually very effective. In addition, ozone is corrosive to some oil and gas materials such as rubber fittings, gaskets and some metal alloys [3]. Generally, oxidizing biocides are associated with some negative side effects such as interaction with other chemicals (corrosion inhibitors), possibility of interaction with non-metallic substances and initiation of corrosion of structural materials [9].

Microbial resistance to biocides can be due to many factors that are related to volume and frequency of application of biocides but most authors link microbial resistance to biocides to formation of biofilms. Biofilms protects sessile bacteria from biocide attacks [10]. Microbial resistance towards biocides could also be as a result of mutation that may arise from frequent use, overdosing or under-dosing (Personal communication). Stoodley et al. [11] showed that dense biofilms with sessile cells glued together by extracellular polymeric substances (EPS) increases mass transfer resistances. The limited nutrition supply decreases the bacterial metabolic activity and increases resistance to biocides. Others suggest that biofilms may change the physiology of sessile bacteria which increases their biocide resistance [10,12].

This paper emanates from a study carried out to demonstrate the level of inhibition of some microbial functional group activities such as the ability to reduce sulfate by sulfate reducing bacteria (SRB), the ability to reduce nitrate by heterotrophic nitrate reducing bacteria (hNRB) and the ability to oxidize sulfide and reduce nitrate by sulfide oxidizing, nitrate reducing

bacteria (so-NRB) by some common oxidizing agents used as biocides such as chlorine, bromine and ozone using both injection and produced water sources. Monitoring resistance with functional group activities of microorganisms is important for the following reasons; 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 sulfide [13]. 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 so-NRB oxidizes sulfide to sulfate with nitrate being reduced to nitrite [14]. Monitoring resistance or tolerance to biocides with functional group activities of microorganisms will therefore be helpful in determining the suitability and efficiency of biocides in controlling bio-corrosion, bio-fouling and oil field reservoir souring.

The main objective was to determine the spectrum of resistance to each biocide in both produced and injection water sources and also to determine if the tolerance or inhibition of the various microbial functional groups activities by the biocides is total or selective.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples used as oxidizing agents such as Chlorine, Ozone and Bromine were obtained from Microcheck Nigeria Limited while the injection and produced water samples were collected from Chevron's Escravos facility, Nigeria.

2.2 Most Probable Number (MPN) Measurement

To quantify the presence of sulfate-reducing bacteria (SRB) in the samples, the API RP-38 broth medium was used. Serial dilution of the samples in API RP-38 broth medium was made with the use of a sterile syringe. 1.0ml of each sample was inoculated to the 9.0ml of the medium and the sequence was repeated serially to the last tube. Samples were then incubated at 37ºC for up to 14 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 medium (Phenol red-dextrose reagent) with a salinity of 5000ppm was used. Change in color from orange to yellow shows the presence of acid producers (Fermentation of dextrose).

2.3 Physicochemical Analysis of Samples

SO_4^{2-} was analyzed with high performance liquid chromatography (HPLC) as described by [15]. Dissolved sulfide was determined using the diamine method [16,17]. NH_4^+ measurement was done using the indole-phenol method while NO_3^-, NO_2^- and organic acids such as acetate, propionate and butyrate were analyzed using HPLC as described in the Standard Methods of [15]. Salinity was measured as Chloride as described in the Standard Methods of [15], while temperature, pH and conductivity were measured with Orion Temp, pH and conductivity meters respectively.

2.4 Microbiological Assay

The medium that was used for the microbiological assay was Coleville synthetic brine (CSB-K) with composition (g/L) as previously described [13]; 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 (1ml), $NaHCO_3$ (1M) 30ml, Na_2S (1M) 1 ml, HCl (2M) 2ml, pH adjusted to 7.4. The Widdel flask was connected to a gas stream of 90% N and 10% CO_2. About 70ml of the medium was then aseptically and anaerobically dispensed to 125ml serum bottles with a gas phase of 90% N and 10% CO_2 and closed with a sterile butyl rubber stopper.

2.5 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) – 40mM lactate and 20mM sulfate; 3mM VFA and 20mM sulphate
b. Heterotrophic nitrate reducing bacteria (hNRB) – 3mM VFA and 10mM nitrate
c. Sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) – 5mM sulfide and 10mM nitrate

Details of the biocide activity test protocol are shown in Table 1.

Table 1. Composition of biocide activity test protocol

	UPW	UIW
Sample Volume in 80ml serum bottle (ml)	25	25
SRB_LS Lactate (mM)	40	40
$S0_4^{2}$(mM)	20	20
SRB_VS VFA (mM)	3	3
$S0_4^{2}$ (mM)	20	20
hNRB VFA (mM)	3	3
NO_3^- (mM)	10	10
So-NRB HS^- (mM)	5	5
$N0_3$- (mM)	10	10
Biocide Conc. (%)	0, 0.1, 0.5, 1	0, 0.1, 0.5, 1
Days Monitored	0, 1, 4, 7, 10, 14	0, 1, 4, 7, 10, 14

UPW=Untreated Produced Water; UIW=Untreated Injection water, VFA= Volatile Fatty Acids

3. RESULTS

3.1 Microbiological and Chemical Constituents of Untreated Produced and Injection Water Samples

The produced and injection water samples used in the study both had relatively high concentrations of SRB and APB (10^5-10^6 and 10^5 - 10^7 cells/ml) respectively. Heterotrophic nitrate reducing bacteria (hNRB) and sulfide oxidizing nitrate reducing bacteria (so-NRB) were also present in both samples. Sulfide, Nitrate and Butyrate were not detected in both samples. Propionate and Acetate were present only in produced water samples which also recorded relatively lower sulfate concentration than the injection water. Detailed results are shown in Table 2.

Table 2. Microbiological and Chemical constituents of untreated produced and injection water samples used in the study

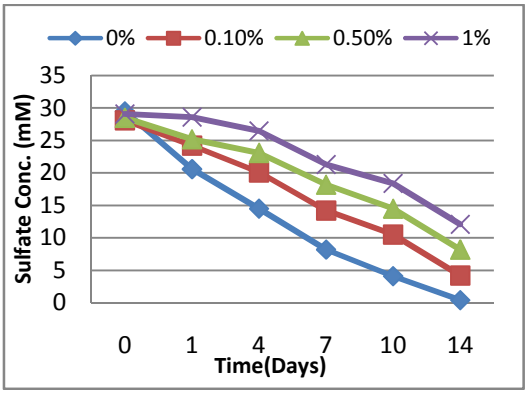
Parameters measured	Untreated Produced water (UPW)	Untreated Injection water (UIW)
SRB (per ml)	10^6	10^5
APB (per ml)	10^7	10^5
hNRB	+	+
so-NRB	+	+
pH	7.1	6.2
HS^- (mM)	0	0
SO_4^{2-} (mM)	11.50	28.50
NH_4+ (mM)	1.40	0.56
NO_3^- (mM)	0	0
NO_2^- (mM)	0	0
Acetate (mM)	4.50	0
Propionate (mM)	1.40	0
Butyrate (mM)	0	0
Salinity (mg/L)	5408	16025
Electrical Conductivity (Ohms)	18.70	26.50

3.2 MPN Counts of SRB and APB In Produced and Injection Water Samples

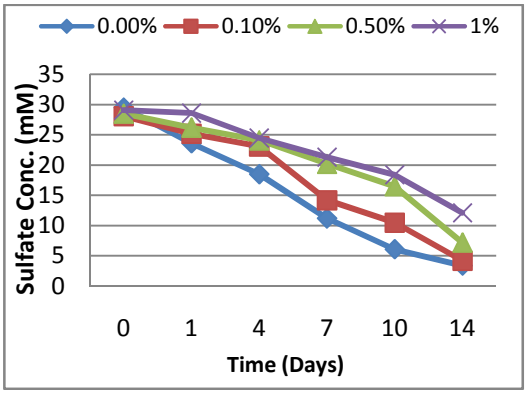
MPN counts in produced and injection water samples showed little inhibition of SRB and APB populations by Chlorine and Bromine even at the highest concentration of 1% used. Only Ozone recorded considerable inhibition at 1% concentration for both SRB (10^6-10^1 cells/ml) and APB (10^7-10^2 cells/ml) after the incubation period of 14 days as shown in Tables 3 and 4.

3.3 Microbial Activities in Untreated Produced Water Samples Incubated with Various Concentrations of Chlorine

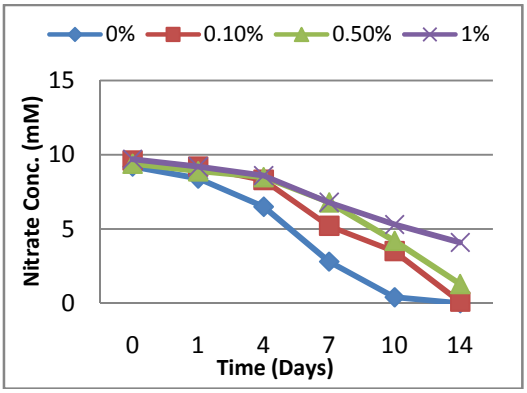
There was an observable resistance of SRB to chlorine in both lactate and VFA media at various concentrations (0-1%) tested going by the rate at which sulfate was reduced by the SRB. Nitrate reduction by the hNRB and the so-NRB were also not inhibited considerably at the highest concentration of 1%, same with the ability of so-NRB to oxidize sulfide as shown in Fig. 1.



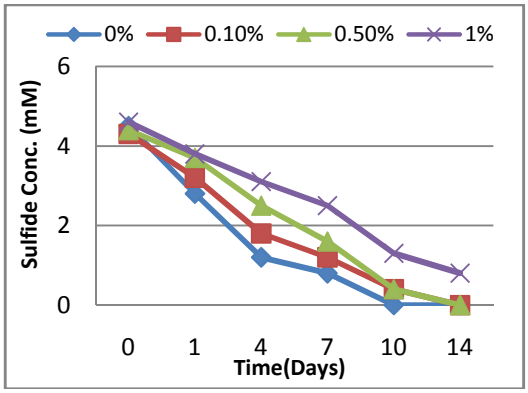
1a. SRB_LS (Sulfate)



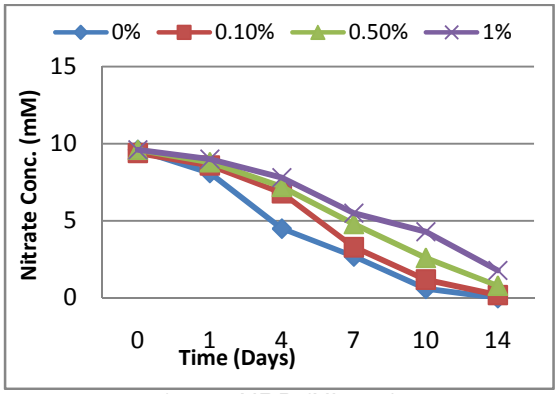
1b. SRB_VS (Sulfate)



1c. hNRB(Nitrate)



1d. so-NRB (Sulfide)



1e. so-NRB (Nitrate)

Fig. 1. Microbial activities in untreated produced water incubated with various concentrations of Chlorine

Table 3. Most Probable Number (MPN) counts of Sulfate reducing bacteria (SRB) and Acid producing bacteria in produced water samples after 2 weeks of incubation with different concentrations of the biocides

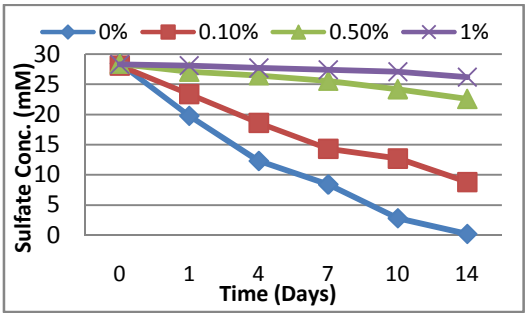
Chlorine (% Conc.)	SRB/ml	APB/ml
0	10^5	10^6
0.1	10^4	10^4
0.5	10^3	10^3
1	10^2	10^3
Ozone (% Conc.)		
0	10^5	10^6
0.1	10^4	10^5
0.5	10^2	10^4
1	10^1	10^2
Bromine (% Conc.)		
0	10^5	10^6
0.1	10^4	10^5
0.5	10^3	10^4
1	10^2	10^3

Table 4. Most Probable Number (MPN) counts of Sulfate reducing bacteria (SRB) and Acid producing bacteria in injection water samples after 2 weeks of incubation with different concentrations of the biocides

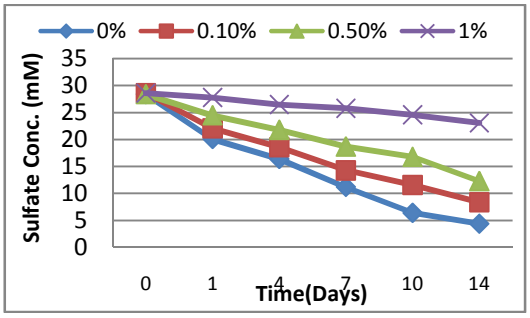
Chlorine (% Conc.)	SRB/ml	APB/ml
0	10^6	10^7
0.1	10^5	10^5
0.5	10^4	10^4
1	10^3	10^3
Ozone (% Conc.)		
0	10^6	10^7
0.1	10^4	10^5
0.5	10^3	10^4
1	10^1	10^2
Bromine (% Conc.)		
0	10^6	10^7
0.1	10^5	10^6
0.5	10^3	10^4
1	10^2	10^3

3.4 Microbial Activities in Untreated Produced Water Samples Incubated With Various Concentrations of Ozone

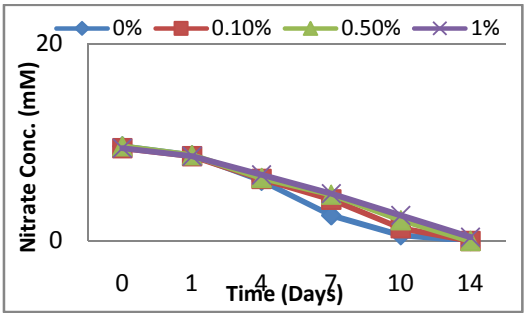
There was an observable inhibition of the rate of sulfate reduction by SRB in both lactate and VFA media at 0.5 and 1% concentration of ozone. The ability of hNRB to reduce nitrate was not considerably inhibited at all the concentrations tested, same with the ability of so-NRB to reduce nitrate but the ability of so-NRB to oxidize sulfide was considerably inhibited at 0.5 and 1% concentration of ozone as shown in Fig. 2.



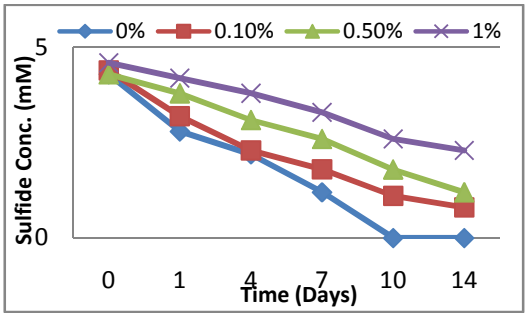
2a. SRB_LS(Sulfate)



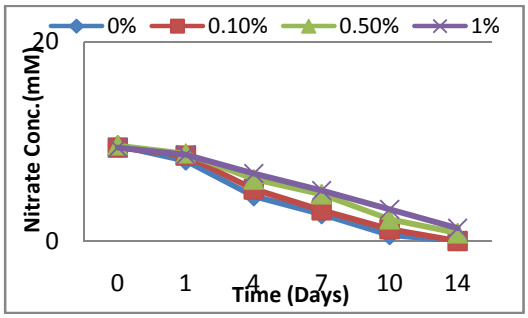
2b. SRB_VS (Sulfate)



2
c. hNRB (Nitrate)



2d. so-NRB (Sulfide)



2e. so-NRB (Nitrate).

Fig. 2. Microbial activities in untreated produced water incubated with various concentrations of Ozone

3.5 Microbial Activities in Untreated Produced Water Samples Incubated With Various Concentrations of Bromine

Bromine did show little inhibition on the ability of SRB to reduce sulfate in both lactate and VFA media. It is same with the ability of hNRB to reduce nitrate and also the ability of so-NRB to oxidize sulfide and reduce nitrate as detailed in Fig. 3.

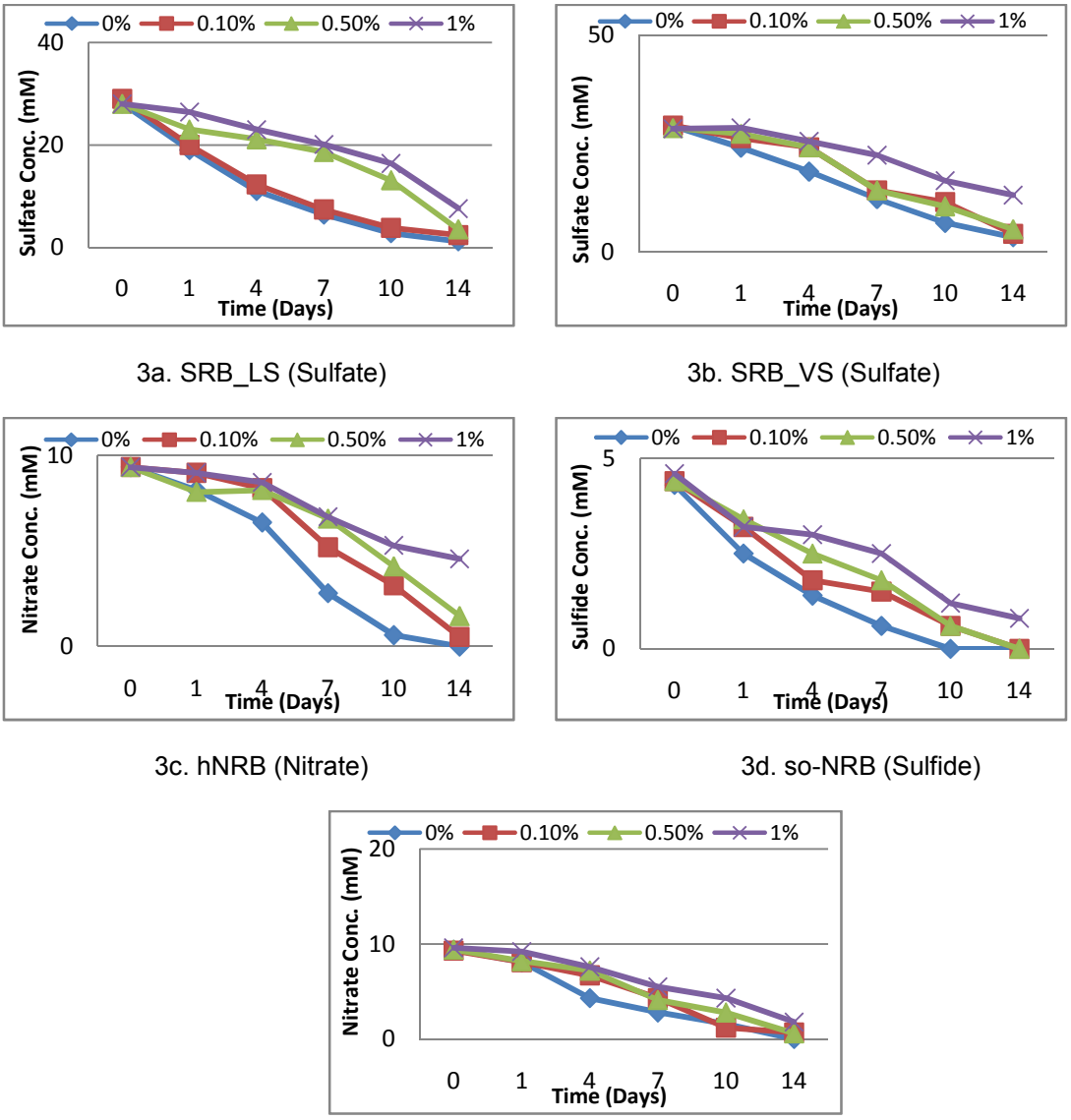
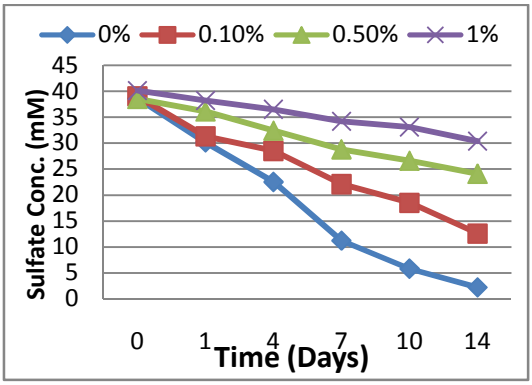


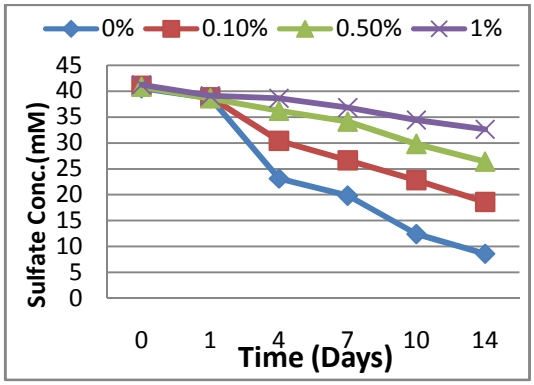
Fig. 3. Microbial activities in untreated produced water incubated with various concentrations of bromine

3.6 Microbial Activities in Untreated Injection Water Samples Incubated With Various Concentrations of Chlorine

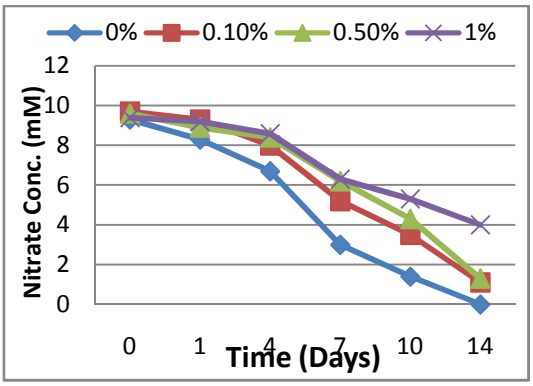
Injection water samples with relatively higher salinity than the produced water recorded an observable inhibition on the ability of SRB to reduce sulfate in both lactate and VFA media at 0.5 and 1% concentration of chlorine. Nitrate reduction by hNRB and so-NRB were not considerably inhibited but the ability of the so-NRB to oxidize sulfide was inhibited considerably at 0.5 and 1% concentration of chlorine as detailed in Fig. 4.



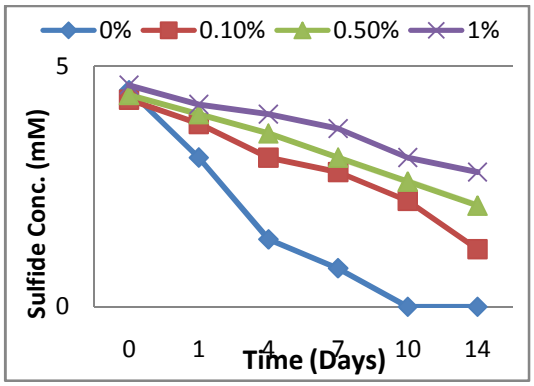
4a. SRB_LS (Sulfate)



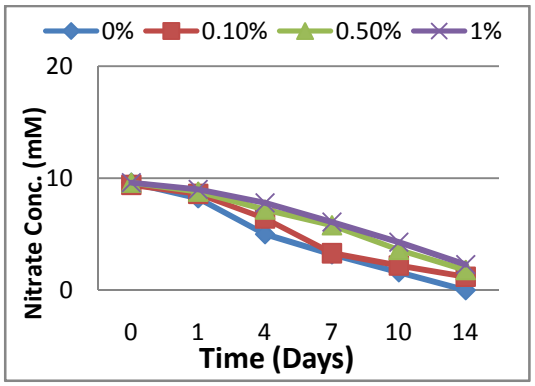
4b. (SRB_LS (Sulfate)



4c. hNRB Nitrate)



4d. so-NRB (Sulfide)

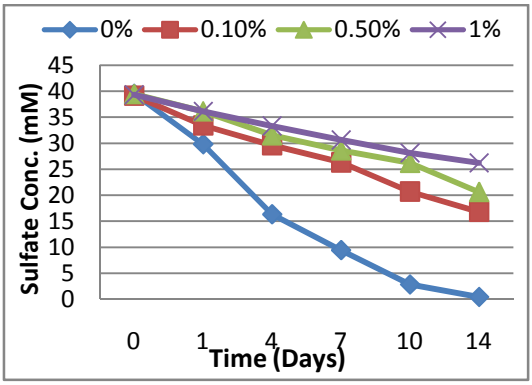


4e. so-NRB (Nitrate)

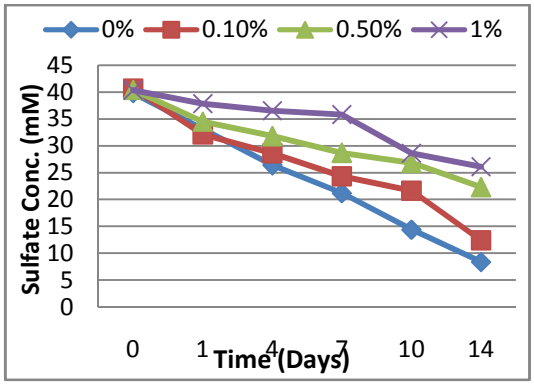
Fig. 4. Microbial activities in untreated injection water (UIW) incubated with various concentrations of chlorine

3.7 Microbial Activities in Untreated Injection Water Samples Incubated With Various Concentrations of Ozone

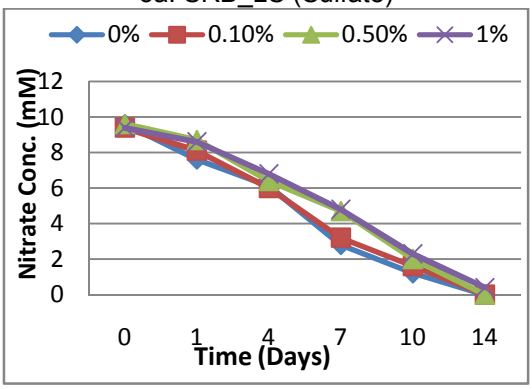
The ability of SRB to reduce sulfate in both lactate and VFA media were considerably inhibited by ozone at 0.5 and 1% concentration. Same with the ability of the so-NRB to oxidize sulfide. Interestingly, the ability of hNRB and so-NRB to reduce nitrate were not inhibited as shown in Fig. 5.



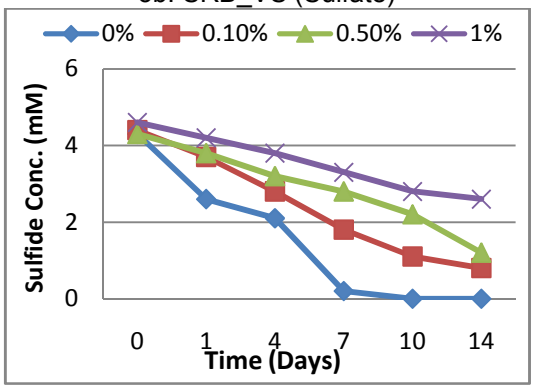
5a. SRB_LS (Sulfate)



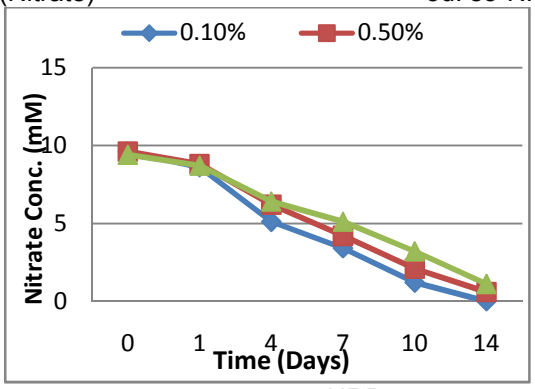
5b. SRB_VS (Sulfate)



5c. hNRB (Nitrate)



5d. so-NRB (Sulfide)



5e. so-NRB

Fig. 5. Microbial activities in untreated Injection Water (UIW) incubated with various concentrations of Ozone

3.8 Microbial Activities in Untreated Injection Water Samples Incubated With Various Concentrations of Bromine

Bromine was moderately effective in the inhibition of the rate of sulfate reduction in both lactate and VFA media at 0.5 and 1% concentration. Nitrate reductions by hNRB and so-NRB as well as sulfide oxidation by so-NRB were not considerably inhibited as shown in Fig. 6.

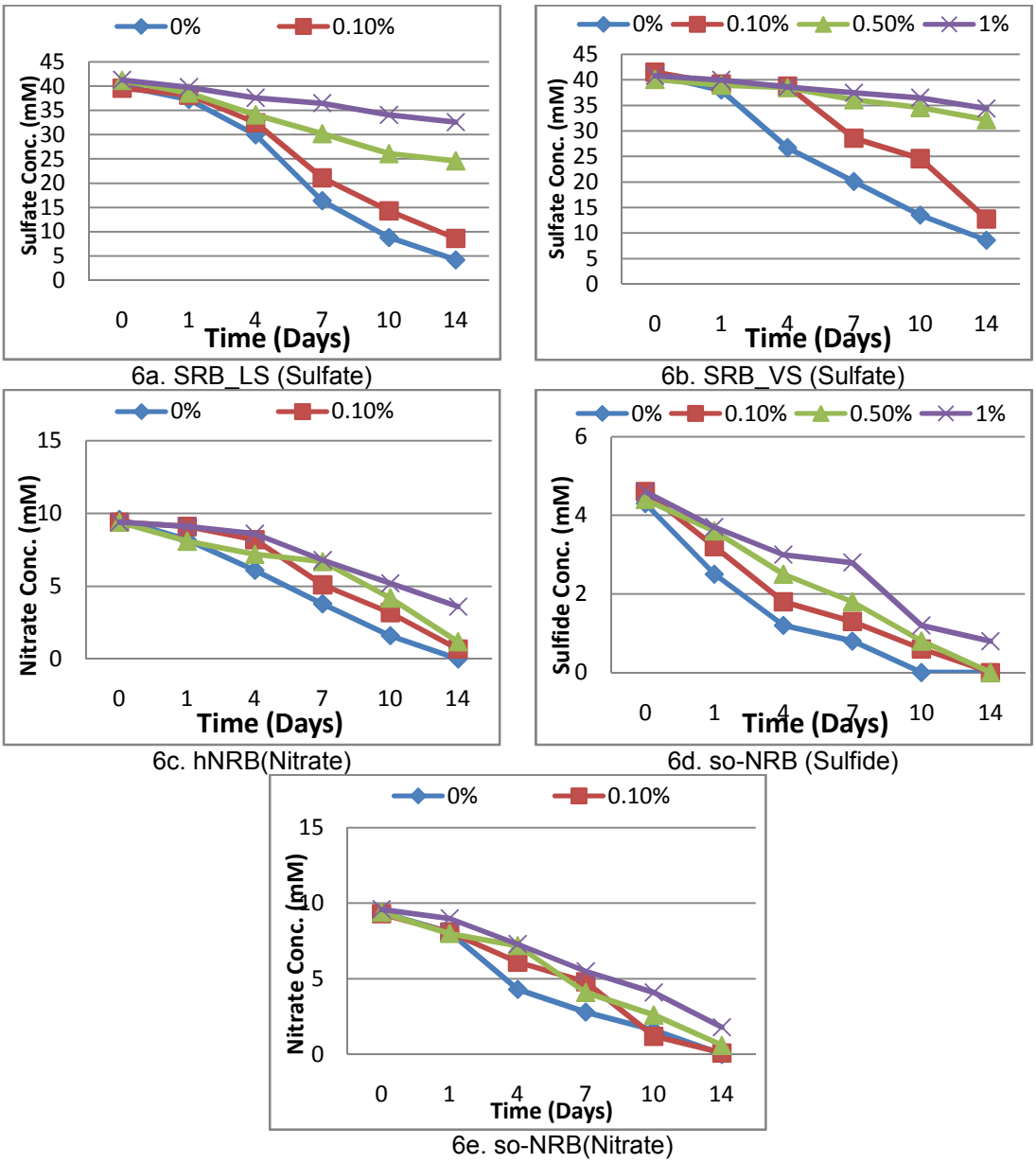


Fig. 6. Microbial activities in untreated injection water (UIW) incubated with various concentrations of bromine

4. DISCUSSION

Functional group activities of microorganisms such as the ability to reduce sulfate, nitrate and the ability to oxidize sulfide and produce hydrogen sulfide were used to determine the extent of resistance or tolerance to the oxidizing biocides used in the investigation. This is a deviation from the traditional methods of monitoring resistance with sessile and planktonic microorganisms [4] and from our literature search, not much has been published on the influence of biocides on the functional group activities of problem causing microorganisms in oil fields.

Using produced water samples, 88.5-98.6% of the original sulfate was reduced by SRB within 14 days the experiment lasted without biocides in both lactate and VFA media but with 1% Chlorine, about 58-58.4% of sulfate reduction was observed. Naturally about 100% of Nitrate was reduced by the hNRB within 14 days but with 1% Chlorine, about 57.7% reduction of nitrate was observed. With so-NRB, 100% of sulfide was oxidized and nitrate reduced within 14 days but when 1% Chlorine was introduced, 82% of sulfide was oxidized while 81.3% of nitrite was reduced.

Comparatively, Ozone achieved about 7.4 and 19.2% (Lactate and VFA media) reduction of sulfate at the highest concentration of 1% while bromine achieved 72.8 and 53.7% reduction of sulfate. On nitrate reduction, about 95.7% of nitrate was reduced by the hNRB while 86.1% was reduced by the so-NRB which also oxidized 50% of sulfide at 1% concentration of ozone. Bromine achieved 51% reduction of nitrate by hNRB and 81.2% reduction by the so-NRB which also oxidized 82.6% of sulfide.

Injection water followed a similar trend with produced water with Ozone showing more efficiency than chlorine and bromine. It was observed that resistance of microbial groups to chlorine was more in injection water than produced water but the reverse was the case with bromine. Comparatively, microbial resistance to the biocides was more common with chlorine and bromine than with ozone in both produced and injection water samples.

With chlorine and bromine, the spectrum of microbial tolerance or resistance to the biocides as it relates to the functional group activities is similar but ozone is somehow more drastic and selective in the sense that the rate of sulfate reduction was far more higher than that of nitrate reduction. Bromine and Chlorine though exhibited some degree of selectivity but that of ozone was more enhanced. The observed selective action might confer some competitive advantage to the hNRB and the so-NRB over the SRB in the utilization of the available organic nutrients [13]. Sulfide oxidation by the so-NRB was considerably inhibited by bromine and chlorine in both produced and injection waters. Recent studies have shown that the three microbial groups used in the present study (SRB, hNRB and so-NRB are abundant in Nigerian oil fields where the studies was carried out [18].

Generally, microbial resistance to all the oxidizing biocides used in the study was observed mostly at lower to moderately higher concentrations (0.1 and 0.5%) in both produced and injection water samples except for ozone that showed considerable inhibition at 0.5%. At the highest concentration of 1%, ozone showed a much higher inhibition on the activity of the functional groups than chlorine and bromine. In addition, some of the oxidizing agents used in the present study like chlorine and bromine make sea water and produced water more corrosive [19,20,21] and their use should therefore be discouraged.

5. CONCLUSION

In conclusion therefore, the use of some oxidizing agents such as chlorine and bromine as biocides for oil field operations should be discouraged because at lower concentrations, they are not efficient and do not have considerable inhibitory activity on the microorganisms that are responsible for souring, fouling and corrosion. Ozone seems to be effective at moderately lower concentrations. More research effort is still needed to see if ozone could be combined with other biocides to improve on its efficiency.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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