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Impacts of Tetrakis-hydroxymethyl Phosphonium Sulfate (THPS) Based Biocides on the Functional Group Activities of Some Oil Field Microorganisms Associated with Corrosion and Souring

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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 biocidal efficacy of THPS based biocides currently used in oil fields to control souring and corrosion.
Methodology: By direct monitoring of inhibition of cell growth and inhibition of microbial functional group activities such as the ability to reduce sulfate and generate sulfide by sulfate reducing bacteria (SRB), reduce nitrate to nitrite by heterotrophic nitrate reducing bacteria (hNRB) and oxidation of sulfide and reduction of nitrate by sulfide oxidizing, nitrate reducing bacteria(so-NRB) using CSB-K medium.
Results: We observed that higher doses of THPS (>400 ppm) was required to considerably inhibit the ability of SRB to reduce sulfate and generate hydrogen sulfide. It was also observed that the activities of SRB were more affected by the THPS biocides than those of hNRB and so-NRB.
Conclusion: We conclude that SRB may have developed low level microbial resistance to THPS based biocides as higher doses are required to inhibit their activities. It is therefore recommended that THPS should be used in combination with other biocides or metabolic inhibitors for it to be effective at lower concentrations.

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Keywords: THPS; Produced water; injection water; functional group activities; SRB; hNRB; so-NRB.

1. INTRODUCTION

Uncontrolled microbial growth in oil field systems can cause severe and costly problems such as souring of oil and gas, microbially influenced corrosion (MIC), formation plugging and biofouling [1,2]. Other negative impacts of poor microbial control in the oil and gas industry include; lost production time, poor quality crude oil and gas and occasional pipeline failures [3]. The most common method of controlling microbial growth in oil fields has been through the application of biocides. While a wide variety of biocides are available for use in oil field systems, improved methods are required to address the problem of efficacy, microbial resistance, economic and environmental concerns [4].

Tetrakis-hydroxymethyl Phosphonium Sulfate (THPS) is one of the most efficient biocides widely used in oil field operations to control oil field reservoir souring, bio-corrosion and biofouling [2,5]. THPSis a water treatment biocide in 75% w/w aqueous solution. It has a broad spectrum activity and it is especially effective against sulfate reducing bacteria (SRB) which are particularly troublesome in enhanced oil recovery operations such as injection water treatment, top-side systems, pipeline protection and storage [5,6]. THPS is widely used in the industry due to its ability to dissolve ferrous sulfide deposits, its effectiveness in both acid and alkaline environments and its low environmental toxicity [5,6]. The structural formula of THPS based biocide used in the present study is shown in Fig. 1.

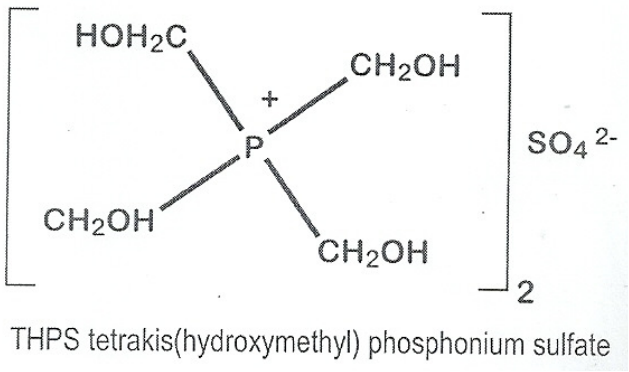


Fig. 1. Structural formula of Tetrakis (hydroxymethyl) phosphonium sulfate (THPS) (Adapted from DOW CHEMICAL COMPANY, 2010)

There has been conflicting reports by several investigators on particular doses of THPS that are ideal for field operations. While Zhao et al. [6] reported a minimum of 50-100 ppm for inhibition of planktonic bacteria and higher doses for established biofilms, Oduola et al. [7] reported THPS doses of about 450 ppm (twice weekly) which was combined with nitrate injection to mitigate SRB. In addition, some investigators have suggested that THPS can be easily degraded to trishydroxy methyl Phosphine oxide (THPO), a process which is facilitated by hydrolysis, oxidation, photodegradation and biodegradation [6]. According to Zhao et al. [6], THPS degradation increases with increased temperature and pH in the presence of mild steel. Other investigators have further suggested that microorganisms had the ability to degrade and use the added biocide for growth [8].

In testing the compatibility of THPS with other biocides, Greene et al. [9] observed that THPS did not exhibit synergy with other biocides like glutaraldehyde, bromopol, formaldehyde and benzakonium chloride. On the contrary, Xu et al. [10] was able to establish a strong synergy between THPS and D-tyrosine for the mitigation of SRB in biofilms.

On the development of an ideal bio-assay that can be used to monitor the efficacy of biocides, Holmkvist et al. [11] suggested a combination of three methods that included direct inhibition of cell growth, monitoring of microbial activity and microbial metabolites such as H_2S and CH_4. Holmkvist et al. [11] however observed that decreased concentration of metabolites in produced water was not directly in agreement with observed increase in the activity of SRB.

Though, THPS is generally regarded as an efficient biocide, it may be possible that microbial resistance to the biocide might have developed over the years, a situation that has prompted industrial application of higher concentrations to mitigate SRB [12].

The main objective of the present contribution therefore was to determine the level of resistance to THPS based biocides by oil field microorganisms especially SRB. The method used to determine the efficacy of the biocide included monitoring of direct inhibition of cell growth and most importantly, monitoring inhibition of functional group microbial activities. The main functional group activities monitored were the inhibition of the ability to reduce sulfate to sulfide by sulfate reducing bacteria (SRB), inhibition of the ability to reduce nitrate to nitrite by the heterotrophic nitrate reducing bacteria (hNRB) and inhibition of the ability to oxidize sulfide and reduce nitrate by the sulfide oxidizing, nitrate reducing bacteria (so-NRB) in produced and injection (sea) water samples as indicated in Fig. 2. Recent studies by Okoro et al. [13] have shown that the three main microbial groups (SRB, hNRB and so-NRB) used in the present investigation are usually present in Nigerian oil fields where the present study was carried out.

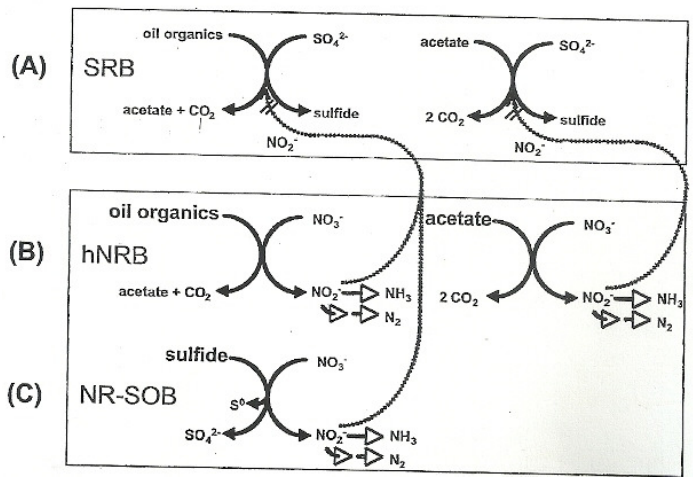


Fig. 2. Functional group microbial activities. (A). SRB couple incomplete oxidation of oil organics to acetate and CO_2 or complete oxidation of acetate to CO_2 to the reduction of sulfate to sulfide.(B). hNRB couple incomplete oxidation of oil organics or complete oxidation of acetate to CO_2 to reduction of nitrate to nitrite and then to either nitrogen or ammonia. (C). NR-SOB (so-NRB) oxidize sulfide to sulfur or sulfate with nitrate being reduced to nitrite and then to either nitrogen or ammonia .(adapted from Voordouw, 2008)

2. MATERIALS AND METHODS

2.1 Sample Collection

THPS sample was obtained from SNEPCO, a subsidiary of Shell Petroleum Development Corporation (SPDC), Nigeria while produced and injection water samples were obtained from Chevron Nigeria Limited.

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 from Dalynn Biologicals was used. Serial dilution of the samples in API RP-38 broth medium was made with the use of a sterile syringe. Each sample (1.0 ml) was inoculated to the 9.0 ml of the medium and the sequence was repeated serially to the last tube. Samples were then incubated at 37℃ 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 (APB), prepared ZPRA-5 medium (Phenol red-dextrose reagent) from Dalynn Biologicals with a salinity of 5000 ppm was used as previously described [13]. 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, WATERS 105, USA) as described by Eaton et al. [14]. Dissolved sulfide was determined using the diamine method of Truper and Schlegel [15]. 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 Eaton et al. [14]. Salinity was measured as Chloride as described in the Standard Methods of Eaton et al. [14], 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 [16]; 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), 2-3 drops of 1% Resazurin. These chemicals were mixed and dissolved in MQ water (Sterile deionized 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.

2.5 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) – 40 mM lactate and 20 mM sulfate; 3 mM VFA and 20 mMsulphate

b. Heterotrophic nitrate reducing bacteria (hNRB) – 3 mM VFA and 10 mM nitrate
c. Sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) – 5 mM sulfide and 10 mM nitrate

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

Table 1. Composition of biocide activity test protocol using THPS at different concentrations

	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.(ppm)	0,200, 400, 600, 800,	0,200, 400, 600, 800,
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 used in the Study

Both samples (untreated injection and produced water) recorded relatively high concentrations of SRB (10^6 and 10^5 cells/ml respectively) and APB (10^8 and 10^7 cells/ml respectively). There was also a considerable presence of hNRB and so-NRB in both samples. Comparatively, sulfate was higher in injection water (28.50 mM) than in produced water (11.50mM), while ammonia was higher in produced water (1.40mM) than in injection water (0.56mM). The detailed results as shown in Table 2 indicated that appropriate microbial groups required for the experiments such as SRB, APB, so-NRB, and hNRB were all present and the environmental conditions were appropriate for their growth and proliferation.

In produced water, THPS completely inhibited SRB growth at a concentration of 400ppm after 2 weeks of incubation, but considerable inhibition was observed at a concentration of 200ppm. In injection water, total inhibition of growth was observed at a concentration of 600ppm (for both SRB and APB) and considerable inhibition was also observed at a concentration of 400ppm (for both SRB and APB) as shown in Table 3.

3.2 Microbial Functional Group Activities in Untreated Produced Water Sample Incubated with Different Concentrations of THPS for 14 days

Under natural experimental conditions and without THPS addition, more than 96% of the original sulfate concentration was reduced by the SRB, about 80% of nitrate was reduced by

hNRB while 100% of sulfide was oxidized and 78% of nitrate reduced by the so-NRB after 2 weeks of incubation in produced water. Addition of 200ppm of THPS did not cause any considerable inhibition on the functional group activities. 400ppm of THPS however affected sulfate reduction by 41-62% in both lactate and VFA media while higher concentrations (600-800 ppm) drastically affected sulfate reduction as the % reduction of sulfate dropped to < 10% after 2 weeks of incubation. Nitrate reduction by the hNRB and sulfide oxidation by the so-NRB also followed a similar pattern as shown in Fig. 3.

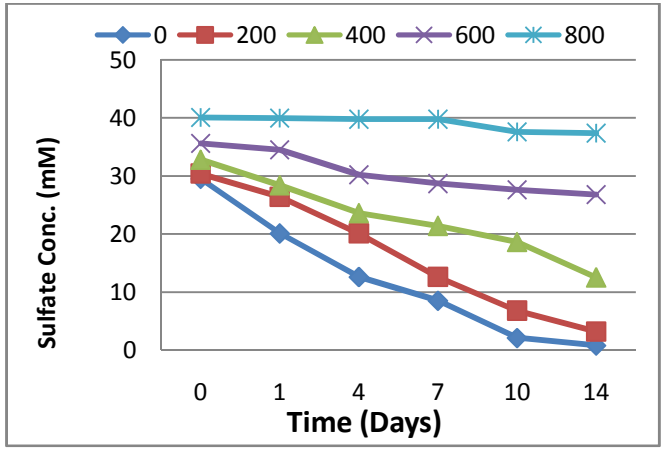
Table 2. Microbiological and Chemical constituents of untreated produced and injection water used in the study

Parameters measured	Untreated Produced water (UPW)	Untreated Injection water (UIW)
SRB (per ml)	10^5	10^6
APB (per ml)	10^7	10^8
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

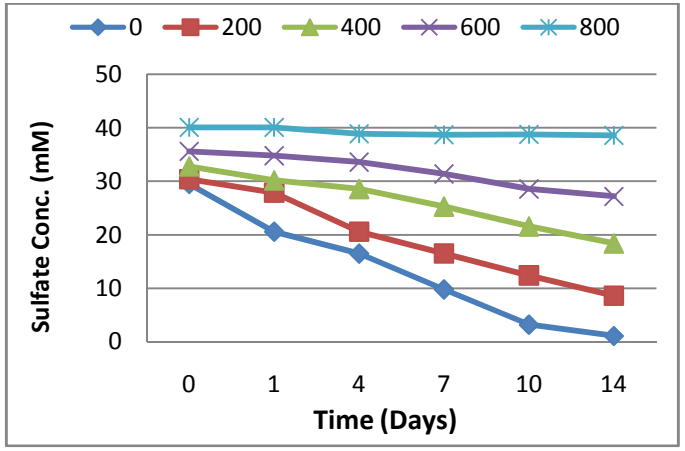
MPN counts of SRB and APB in untreated produced and injection water samples after 2 weeks of incubation with different concentrations of biocides

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

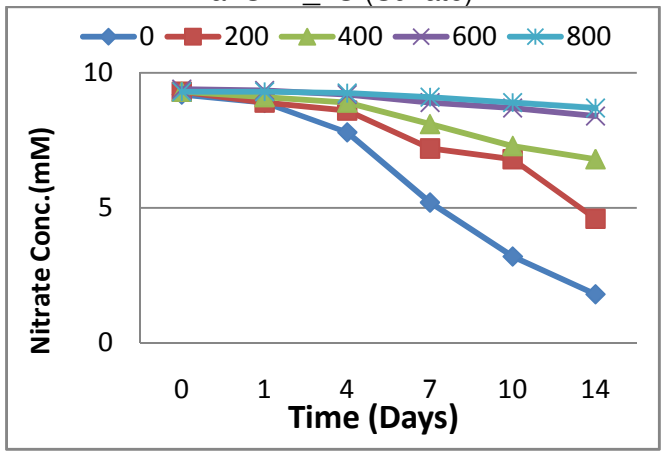
Produced water		
THPS Conc. (ppm)	**SRB/ml**	**APB/ml**
0	10^5	10^7
200	10^2	10^4
400	10^0	10^1
600	10^0	10^0
800	10^0	10^0
1000	10^0	10^0
Injection water		
THPS Conc. (ppm)	**SRB/ml**	**APB/ml**
0	10^6	10^8
200	10^4	10^6
400	10^2	10^3
600	10^0	10^0
800	10^0	10^0
1000	10^0	10^0



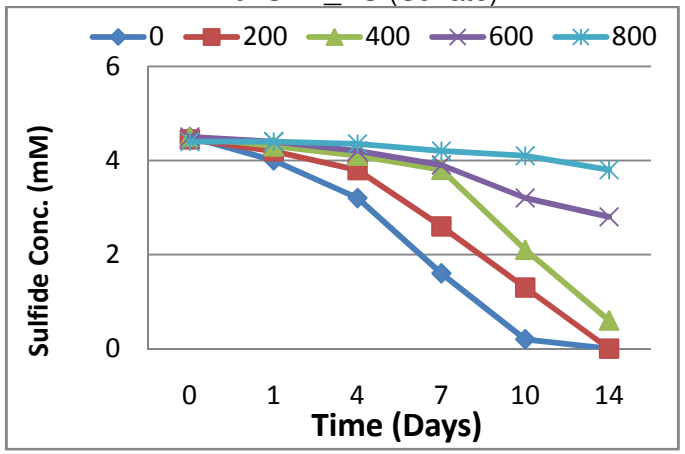
1a. SRB_LS (Sulfate)



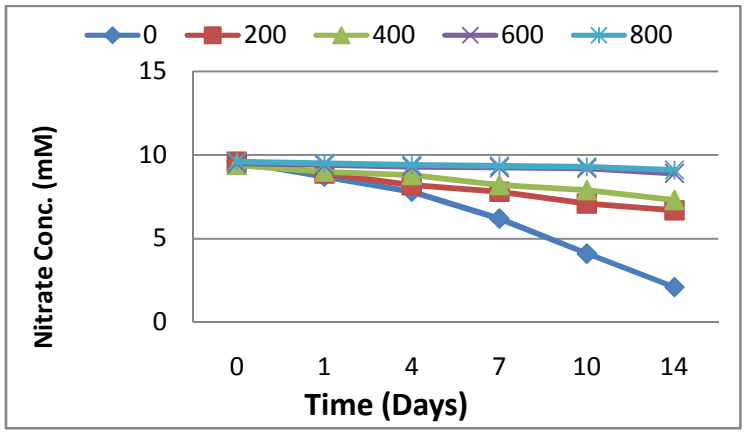
1b. SRB_VS (Sulfate)



1c. hNRB (Nitrate)

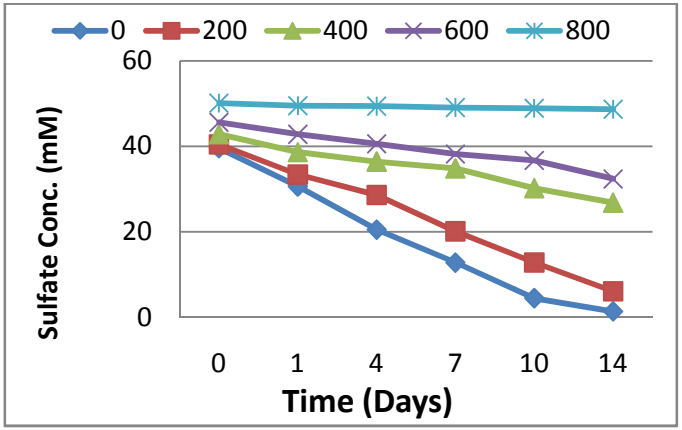


1d. So-NRB (Sulfide)

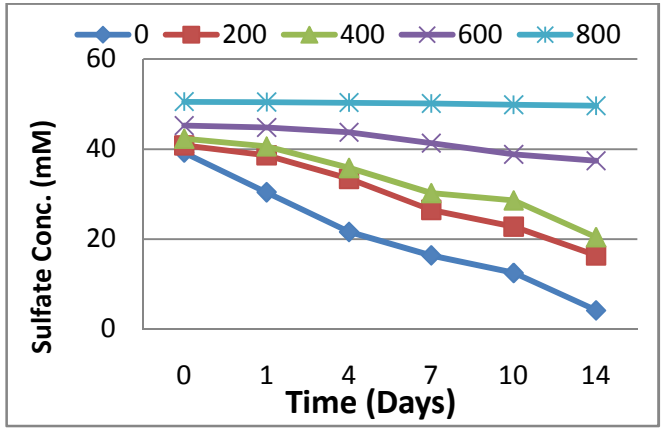


1e. So-NRB (Nitrate)

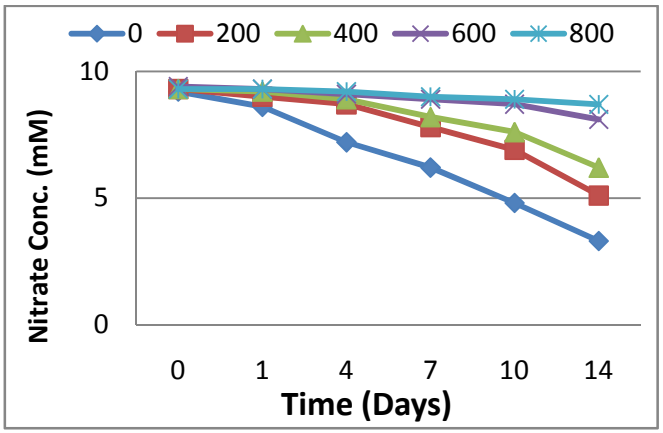
Fig. 3. Microbial activities in untreated produced water sample incubated with various concentrations of THPS (ppm)



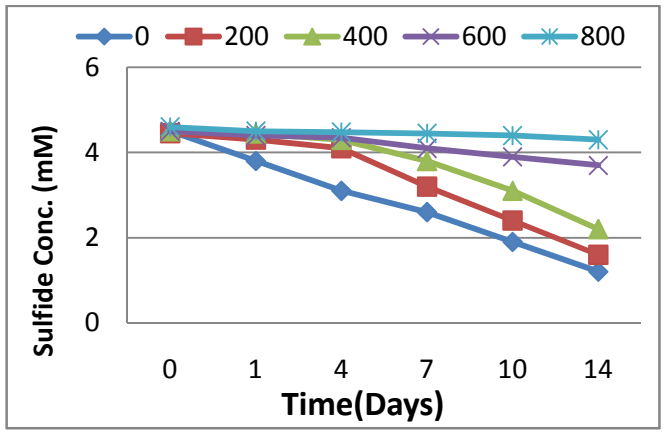
2a. SRB_LS (Sulfate)



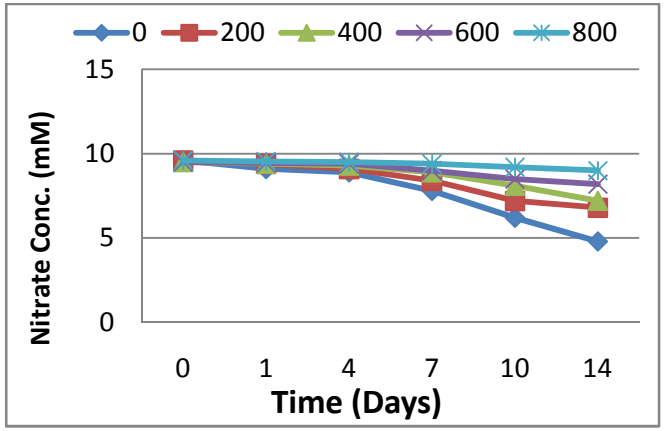
2b. SRB_VS (Sulfate)



2c. hNRB (Nitrate)



2d. So-NRB (Sulfide)



2e. So-NRB (Nitrate)

Fig. 4. Microbial activities in untreated injection water sample incubated with various concentrations of THPS (ppm)

3.4 Microbial Functional Group Activities in Untreated Injection Water Sample Incubated with Different Concentrations of THPS for 14 days

Under natural environmental conditions and without THPS addition, about 90% of the original sulfate concentration was reduced by the SRB, 64% of original nitrate concentration was reduced by the hNRB while about 73% of sulfide was oxidized and 50% of nitrate reduced by the so-NRB. Addition of 200 ppm of THPS did not cause any considerable inhibition of functional group activities as was also observed in produced water. Incubation with 400 ppm THPS affected sulfate reduction rate by 40-50% in both lactate and VFA media while higher concentrations of THPS (600-800 ppm) considerably inhibited sulfate reduction rate. The ability to reduce nitrate to nitrite by hNRB, and oxidize sulfide by the so-NRB were also inhibited considerably at higher THPS concentrations (600-800 ppm) as shown in Fig. 4 above. Comparatively, it was noted that the rate of sulfate reduction by the SRB, nitrate reduction by the hNRB and sulfide oxidation by the so-NRB were higher in produced water than in the injected seawater.

4. DISCUSSION

MPN results showed that THPS inhibited SRB growth completely at a concentration of 400 ppm in produced water while injection water required higher concentration (600 ppm) for complete SRB inhibition after 2 weeks of incubation. It was however observed that considerable inhibition was achieved with 200 ppm of THPS in produced water and 400 ppm in injection water. Dow chemical company [17] had recommended an intermittent dosage of 93-350 ppm (2-6 hrs) and a weekly maintenance dose of 14-67 ppm for injection water treatment (produced and sea water). Miller et. al. [18] reported that a weekly 4hr dose of 150 mg/l of THPS inhibited bio-fouling formation around an injection well while Zhao et. al. [19] recommended a dosage of 50-100 ppm for SRB inhibition.

The present study however revealed that inhibition of microbial functional group activities like the ability to reduce sulfate by SRB, reduce nitrate by hNRB, oxidize sulfide and reduce nitrate by the so-NRB required higher doses of THPS (>400 ppm) for considerable inhibition. Our findings seemed to suggest that THPS was not usually very effective at lower to medium range doses (200-400 ppm), an indication of a probable low level microbial resistance. Application of THPS based biocides at higher concentrations may not be ideal because apart from the cost implication, the biocides may cause corrosion if applied at high concentrations [20] and might possibly kill community members that offer protection against corrosion [21]. Another interesting observation was that the level of resistance to THPS by SRB was higher than what was observed with other microbial communities like the hNRB and so-NRB. Reverse sample genome probing (RSGP) analysis of SRB enrichments had shown that organisms present on corrosion coupons such as the SRB are often more resistant to the biocides used for their control than other community members [22].

Practical application of THPS in the field is usually done in combination with other metabolic inhibitors of SRB such as nitrite. Oduola et al. (7) for instance reported that for the control of souring at Bonga deep offshore Nigeria operated by SNEPCO, THPS was injected at a treatment rate of 450 ppm for 1hr. (twice weekly dosage) in combination with calcium nitrate. The purpose of nitrate injection was to stimulate the activities of hNRB which compete with SRB for organic nutrients. Since THPS also inhibit the activities of hNRB and so-NRB though at higher concentrations, addition of nitrite to directly inhibit SRB activities seemed to be a better option. Some other investigators [23,24] had observed that nitrite which is a

product of nitrate reduction by hNRB and so-NRB is a powerful SRB inhibitor and nitrite have worked efficiently with some biocides to inhibit SRB activities.

In testing the compatibility of THPS with other biocides, Green et al. [9] observed that THPS did not exhibit synergy with other biocides like glutaraldehyde, bromopol, formaldehyde and benzakonium chloride but most recently, Xu et al., [10] reported that strong synergy existed between THPS and D-tyrosine combination for mitigation of SRB in biofilms. For instance, it was found that when 100 ppm of D-tyrosine and 50 ppm of THPS were both used alone and individually, they were found to be ineffective against SRB biofilm but when 1ppm of D-tyrosine was combined with 50 ppm of THPS, the synergy between the two successfully prevented the establishment of an SRB biofilm. The major challenge of this combination however is the cost implications of developing D-tyrosine and making it available at commercially industrial quantities.

5. CONCLUSION AND RECOMMENDATION

The present study has demonstrated that THPS may have developed some low level microbial resistance over the years since higher doses (>400ppm) are required to considerably inhibit the ability of SRB to reduce sulfate and generate hydrogen sulfide. The study also showed that the injected sea water required higher concentrations of THPS than produced water to inhibit SRB activities. Future research effort should therefore be focused on the development of an ideal THPS biocide that is tolerated by the hNRB and so-NRB while inhibiting SRB at relatively lower concentrations. The THPS biocide should also be able to work in synergy with a relatively cheap and commercially available metabolic inhibitor for proper mitigation of souring and bio-corrosion.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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