British Microbiology Research Journal
4(10): 1118-1137, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Impacts of Some Non-oxidizing Biocides on the Functional Group Activities of Some Problem Causing Microorganisms in Oil Production Facilities

Okoro Chuma Conlette[1*]

[1]*Petroleum Microbiology Research Unit, Department of Biology, Microbiology and Biotechnology Federal University, Ndufu Alike-Ikwo, Ebonyi State, Nigeria.*

Author's contribution

This whole work was carried out by author OCC.

Original Research Article

Received 13th March 2014
Accepted 14th May 2014
Published 16th June 2014

ABSTRACT

Aim: To determine the impacts of some non-oxidizing biocides such as glutaradehyde, sodium azide, isothiazolone on the functional group activities of some oil field microorganisms
Methodology: Samples of non-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: Glutaradehyde and sodium azide exhibited relatively high level inhibition while isothiazolones exhibited low level inhibition. Glutaradehyde further demonstrated a positive selective inhibitory activity. While SRB activities were inhibited by over 78%, that of hNRB and so-NRB were affected by less than 38%.
Conclusion: Glutaradehyde can be developed to an efficient biocide with a positive selective action and can work in synergy with beneficial microbes to eliminate the problem causing ones.

Keywords: Glutaradehyde; sodium azide; isothiazolone; srb; hnrb; so-nrb; non-oxidizing biocides.

**Corresponding* author: *Email: Chuma2k2001@yahoo.com;*

1. INTRODUCTION

Biocides are chemical compounds used to disinfect, decontaminate and sterilize materials (surfaces or objects) in order to eliminate microbiological degradation processes [1]. The mode of action of biocides is to stop the current metabolic activity of the microorganism, causing changes in the proper functioning of cells and consequently death of the microorganism [2] Researchers [3,4] observed that the effectiveness of the biocide depends on several factors which include the concentration, duration of contact and water quality variables, temperature, pH, turbidity, organic matter and dissolved solids. Generally, biocides are used in all stages of oil field development, from the initial drilling of wells, during production of oil and gas and all aspects of maintenance of field operations including storage of oil and natural gas [5,6].

Given the mechanism of chemical action of biocides, biocides can be divided into two groups, i.e. those that make use of oxidizing agents such as chlorine, ozone and bromide and those that use non-oxidizing agents such as enzyme inhibitors and protein denaturants (Glutaradehyde, Sodium azide and Isothiazolone) or surface agents such as quaternary ammonium compounds. According to Klechka [7], biocides used in oil and gas operations should meet the following requirements.

i. They should be effective at low concentrations (10-100ppm)
ii. They should have high biocidal capabilities and broad spectrum of activity.
iii. Long duration of action
iv. Good solubility in water and hydrocarbons
v. Chemical and thermal stability
vi. Environmental friendliness and easy biodegradability
vii. Economical and cost effective
viii. Lack of side effects such as corrosion and destruction of catalysts used in oil processing.

In the present study, we are looking at the impacts of some non-oxidizing biocides that inhibit microbial enzymes and denature proteins such as glutaradehyde, sodium azide and isothiazolone on the functional group activities of oil field microorganisms such as the ability to reduce sulfate, nitrate and oxidize sulfide. Gluteradehyde is a widely used biocide in oil fields because of its broad spectrum nature and biodegradability. Due to its ability to denature proteins, gluteradehyde is also used a fixative [8]. Azides are energy rich molecules with many applications. Sodium azide for example is used as a preservative, mutagen, biocide and assay reagent. In some Nigerian oil fields, low corrosion rates have been reported in pipelines treated with sodium azide [9]. Isothiazolone biocides have proven efficacy and performance for microbial control in a variety of industrial water treatment applications. They utilize a 2-step mechanism involving rapid inhibition within minutes of growth and metabolism followed by irreversible cell damage resulting in loss of viability [10]. Some researchers have advocated that environmental condition containing hydrogen sulfide cause deactivation of isothiazolones [5].

Microbial resistance to biocides can be due to so many factors that are related to volume and frequency of application of biocides but some researchers link microbial resistance to biocides to formation of biofilms. Biofilms protects sessile bacteria from biocide attacks [11]. Stoodley and colleagues [12] 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 [13,11].

The main goal of the present contribution is to determine the impact of some powerful non oxidizing biocides on 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). Apart from the functional group activities, the effect of the biocides on acid producing bacteria was also monitored. Acid producing bacteria (APB) partially oxidize hydrocarbons to form organic acids, they are the first to colonise metal surfaces during the initiation of biofilms. They are facultative anaerobes and thrive under virtually all conditions. The organic acids they produce such as acetic acid, formic acid and propionic acid causes very acidic conditions to develop beneath the colony and some biocides are known to be inactivated under very acidic conditions [5].

The study was carried out using both injection and produced water sources. The aim 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 was total or selective. This we hope will give further insights into the control of some microbial related problems in the oil industry such as bio-corrosion, bio-fouling and oil field reservoir souring.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of the non-oxidizing agents such as sodium azide, isothiazolone and gluteradehyde 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 9.0ml of the medium and the sequence was repeated serially up to the last tube. Samples were then incubated at 37ºC 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 medium (Phenol red-dextrose reagent) with a salinity of 5000 ppm was used. Change in color from orange to yellow shows the presence of acid producers (Fermentation of dextrose).

1.3 Physicochemical Analysis of Samples

Sulfate (SO_4^{2-}) was analyzed with high performance liquid chromatography (HPLC) as described by Eaton et al. [14]. Dissolved sulfide was determined using the diamine method [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 [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.

1.4 Microbiological Assay

The medium that was used for the microbiological assay was Coleville synthetic brine (CSB-K) with composition (g/L) as previously described [8]; 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) and 2-3 drops of Resazurin (1%). 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 (1.0ml), Selenate-tungstate (1.0ml), $NaHCO_3$ (1.0 M) 30ml, Na_2S (1.0M) 1ml, HCl (2.0M) 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 compositions of electron donor and acceptor 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

Sample Volume in 80ml serum bottle (ml)	**UPW**	**UIW**
	25	**25**
SRB_LS		
Lactate (mM)	40	40
$S0_4^{2-}$(mM)	20	20
SRB_VS		
VFA (mM)		
$S0_4^{2-}$ (mM)	3	3
	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, 0.7, 1	0, 0.1, 0.5, 0.7, 1
Days Monitored	0, 1, 4, 7, 10, 14	0, 1, 4, 7, 10, 14

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 (10^7 and 10^6 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 (26.80mM) than in produced water (13.50mM), while ammonia was higher in produced water (1.44mM) than in injection water (0.5mM). The detailed results as shown in table 2 indicated that appropriate microbial groups required for the experimentation such as SRB, APB, so-NRB, and hNRB were all present and the environmental conditions were appropriate for their growth and proliferation.

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)	10^7	10^6
APB (per ml)	10^8	10^7
hNRB	+	+
so-NRB	+	+
pH	7.1	6.2
HS^- (mM)	0	0
SO_4^{2-} (mM)	13.50	26.50
NH_4+ (mM)	1.44	0.50
NO_3^- (mM)	0	0
NO_2^- (mM)	0	0
Acetate (mM)	4.20	0
Propionate (mM)	1.30	0
Butyrate (mM)	0	0
Salinity (mg/L)	5508	14020
Electrical Conductivity (Ohms)	17.60	24.50

2.2 MPN Counts of SRB and APB in Untreated Produced Water Samples after 2 Weeks of Incubation with Different Concentrations of Biocides

Sodium azide and Glutaradehyde at 1% concentration showed complete inhibition of SRB and APB growth. At a lower concentration (0.5%), glutaradehyde showed considerable inhibition of SRB (10^7-10^1) and APB (10^8-10^2). Sodium azide also showed considerable but lower inhibitory capability when compared with glutaradehyde. Comparatively, isothiazolone did not show complete inhibition of SRB and APB at 1% concentration as with the other biocides. Detailed results are shown in Table 3.

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

Sodium azide (%Conc.)	SRB/ml	APB/ml
0	10^7	10^8
0.1	10^4	10^5
0.5	10^2	10^3
1	10^0	10^0
Isothiazolone (% Conc.)		
0	10^7	10^8
0.1	10^5	10^6
0.5	10^3	10^4
1	10^1	10^2
Glutaradehyde (% Conc.)		
0	10^7	10^8
0.1	10^3	10^4
0.5	10^1	10^2
1	10^0	10^0

2.3 MPN Counts of SRB and APB in Untreated Injection Water Samples after 2 Weeks of Incubation with Different Concentrations of Biocides

Complete inhibition of SRB and APB growth was observed at 1% concentration in injection water samples with sodium azide and glutaradehyde. Glutaradehyde also achieved complete inhibition of SRB at lower concentrations (0.5%). Microbial inhibition at 1% Isothiazolone was also considerable but not total when compared with sodium azide and glutaradehyde as shown in Table 4.

2.4 Functional Group Microbial Activities in Untreated Produced Water Samples Incubated With Different Concentrations of Glutaradehyde for 14 Days

Under natural conditions, SRB reduced about 96% of sulfate originally present in lactate media and 85% in VFA media within 14 days of incubation. With various concentrations of glutaraldehyde (0.5, 0.7 and 1%), the following respective % reduction of sulfate was observed in lactate media (22, 9.8 and 3.7%) and VFA media (15, 7 and 2%) within 14 days. As opposed to SRB, the activities of the hNRB were not drastically affected by the biocides as about 63% nitrate reduction was observed even at higher concentration (1%) within 14 days of incubation. The activities of the so-NRBs that oxidizes sulfide and reduces nitrate were also not drastically affected by the biocides. About 52.3% of the sulfide was oxidized while 77.7% of nitrate reduced after 14 days of incubation with 1% glutaradehyde. Detailed results are shown in Fig. 1.

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 concentration of the biocides

Sodium azide (% Conc.)	SRB/ml	APB/ml
0	10^6	10^7
0.1	10^4	10^5
0.5	10^1	10^2
1	10^0	10^0
Isothiazolone (% Conc.)		
0	10^6	10^7
0.1	10^5	10^6
0.5	10^3	10^4
1	10^1	10^2
Gluteraldehyde (% Conc.)		
0	10^6	10^7
0.1	10^3	10^4
0.5	10^0	10^1
1	10^0	10^0

2.5 Functional Group Microbial Activities in Untreated Produced Water Samples Incubated With Different Concentrations of Sodium Azide for 14 Days

Under natural conditions, SRB in produced water reduced about 95% of sulfate originally present in lactate media and 80% in VFA media after 14 days. With lower concentrations of sodium azide (5%), about 30% of sulfate was reduced in lactate media while 9.3% was reduced in VFA media. At higher concentrations (0.7 and 1%), the respective % reduction in lactate media were 17 and 11 % while VFA recorded 4.4 and 2.8% respectively. This is an indication of a biocide with a strong inhibitory capability. Without the biocides, 100% of nitrate was reduced by hNRB after 14 days but with various concentrations of biocides (0.5, 0.7 and 1%), the respective % reduction of nitrate observed were 35.4, 19.6 and 3.1. This is an indication that the activities of the hNRBs are stongly inhibited at higher concentrations (0.7, and 1%). In contrast however, the capabilities of the so-NRB to oxidize sulfide and reduce nitrate were not considerably inhibited even at higher concentrations as shown in Fig. 2.

2.6 Functional Group Microbial Activities in Untreated Produced Water Samples Incubated With Different Concentrations of Isothiazolone for 14 Days

Without the biocides, about 92% of the original sulfate concentration was reduced by SRB in lactate media while 71% was reduced in VFA media after 14 days. With is othiazolone concentrations of 0.5, 0.7 and 1%, the respective % reduction of the original sulfate concentration by the SRB in lactate media were 54.4, 37.8 and 15.6% while VFA media recorded 34, 21.5 and 4.1% respectively. hNRB activities were inhibited considerably at higher concentrations (0.7 and 1%) where the % nitrate reduction recorded were 38 and 16.8% respectively. Same scenario followed sulfide oxidation and nitrate reduction by the so-NRB where the respective % sulfide oxidation and nitrate reduction were 37.8, 27.3% and 25, 13.8% respectively. Detailed results are shown in Fig. 3.

2.7 Functional Group Microbial Activities in Untreated Injection Water Samples Incubated With Different Concentrations of Glutaradehyde for 14 Days

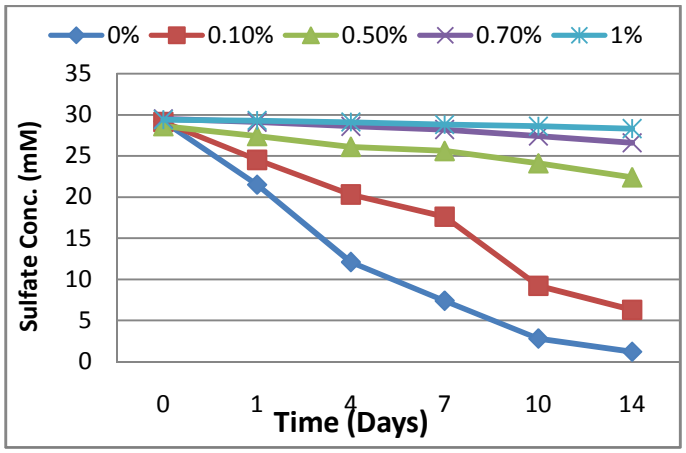
Without glutaradehyde, about 90% of the original sulfate concentration was reduced in lactate media while 67% was reduced in VFA media after 14 days. It was noted that 67% reduction of sulfate by the SRB in VFA media as opposed to 90% in lactate media might be due to preference of lactate over VFA and not necessarily due to the presence of inhibitory substances. The respective % reduction of sulfate by SRB in lactate media at different concentrations (0.5, 0.7 and 1%) of glutaradehyde were 5, 1.9 and 0.7 % while VFA media recorded 4.8, 2.5 and 1.4%. This is a strong indication of inhibition of sulfate reduction at lower concentrations (0.5%). Nitrate reduction by the hNRB were considerably inhibited at higher concentrations (0.7 and 1%) with respective % nitrate reduction values (16.5 and 12.5%). On the contrary, the activities of the so-NRB were not considerably inhibited by the biocides even at higher concentrations as shown in Fig. 4.

2.8 Functional Group Microbial Activities in Untreated Injection Water Samples Incubated With Different Concentrations of Sodium Azide for 14 Days

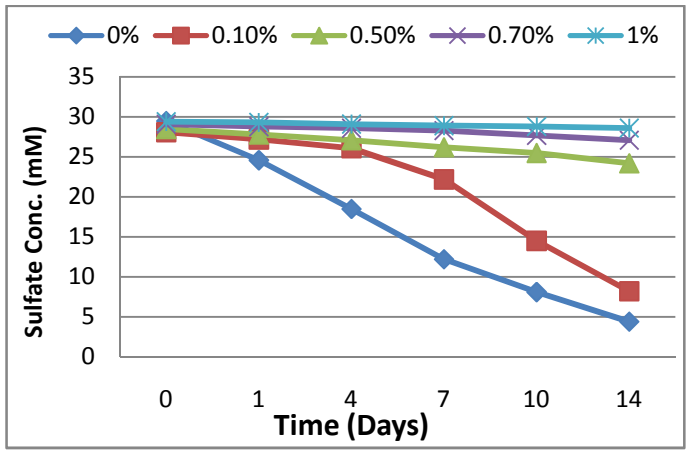
The respective % sulfate reduction without the biocide in lactate and VFA media were 94.7 and 71.5 %. At various concentrations of sodium azide (0.5, 0.7 and 1.0%), the respective % reduction in lactate were 5.2, 2.8 and 0.7 while VFA media recorded 4.8, 2.3 and 1.6 respectively. The activities of the hNRB were not considerably inhibited even at higher concentrations and similarly with so-NRB activities as shown in Fig. 5.

2.9 Functional Group Microbial Activities in Untreated Injection Water Samples Incubated With Different Concentrations of isothiazolone for 14 Days

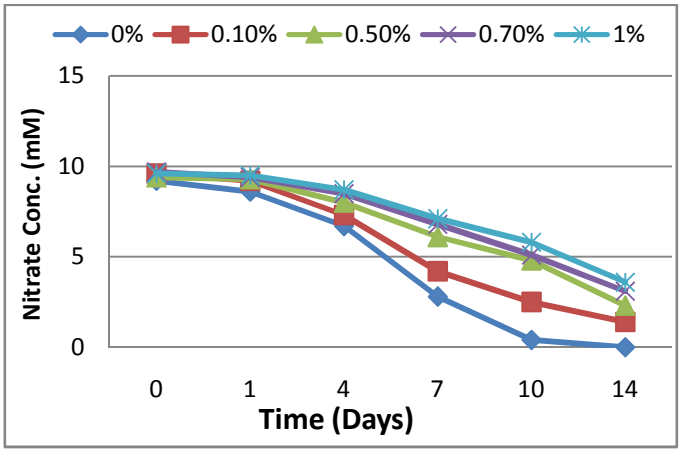
Without isothiazolone, about 95% of the original sulfate concentration was reduced in lactate media while 73.8% was reduced in VFA media. With various concentrations of isothiazolone (0.5, 0.7 and 1.0), the respective % reduction of sulfate in lactate media were (52, 48.6 and 33.6) while VFA media recorded 30.2, 25.3 and 6.2 respectively. Nitrate reduction was considerably inhibited at higher concentrations (0.7 and 1.0%). The same scenario was observed in so-NRB activities as shown in Fig. 6.



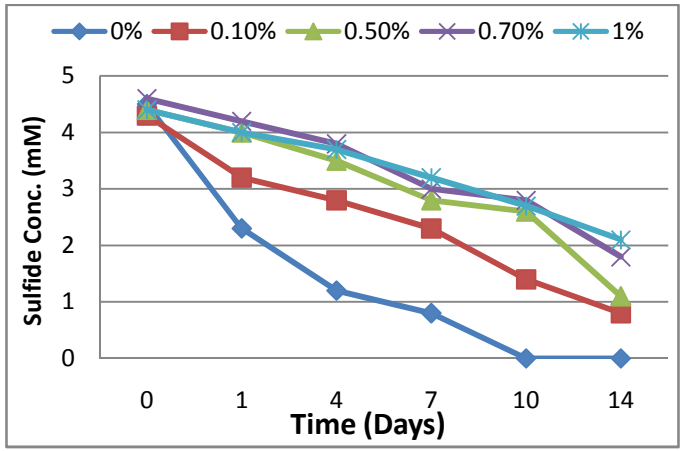
1a. SRB_LS (Sulfate)



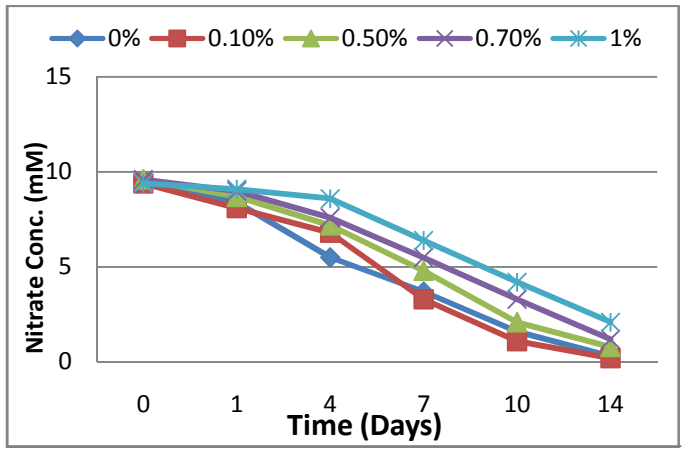
1b. SRB_VS (Sulfate)



1c. hNRB (Nitrate)

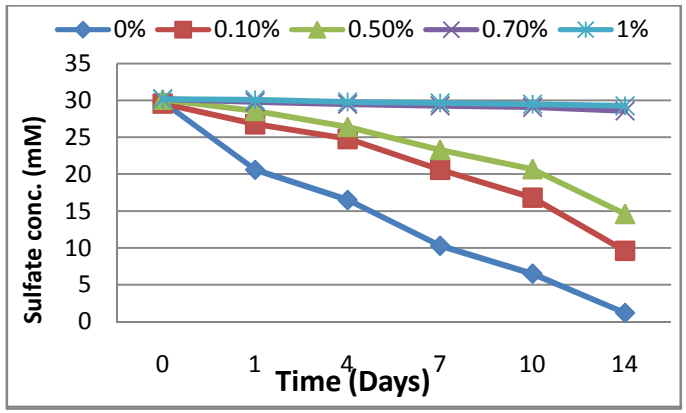


1d. so-NRB (Sulfide)

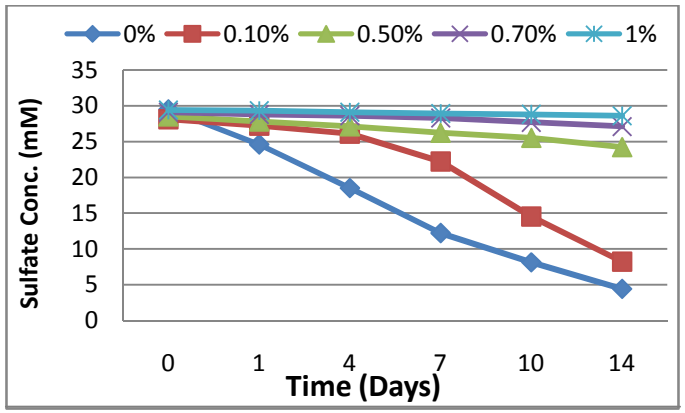


1e. so-NRB (Nitrate)

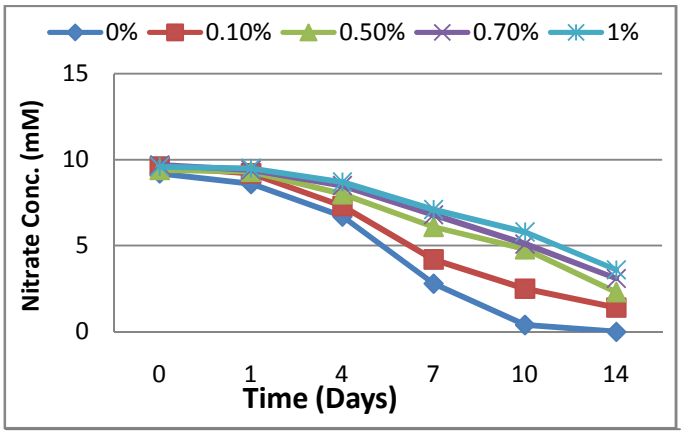
Fig. 1. Microbial activities in untreated produced water sample incubated with various concentrations of gluteraldehyde for 14 days



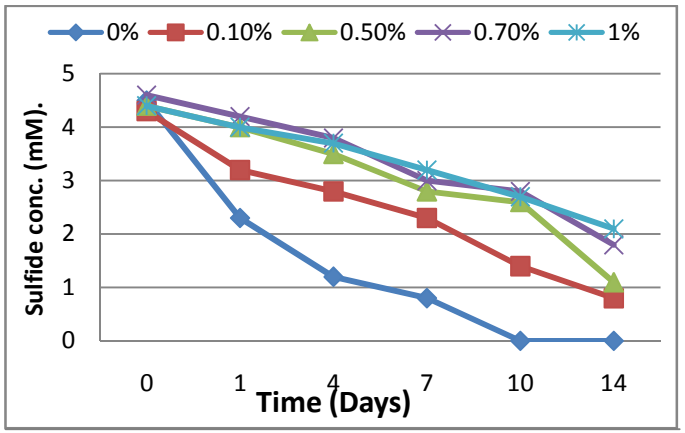
2a. SRB_LS (Sulfate)



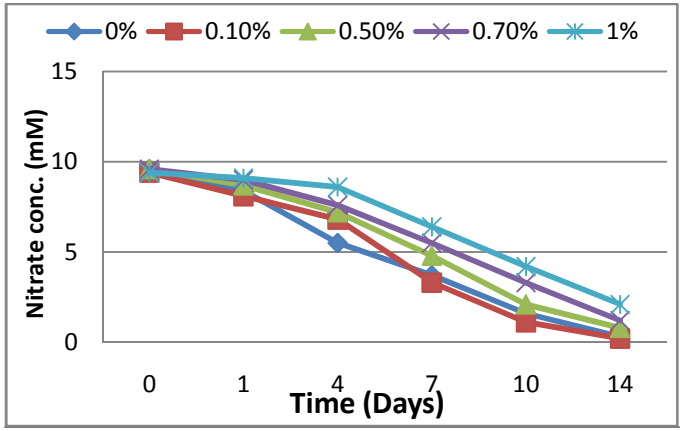
2b. SRB_VS (Sulfate)



2c. hNRB (Nitrate)

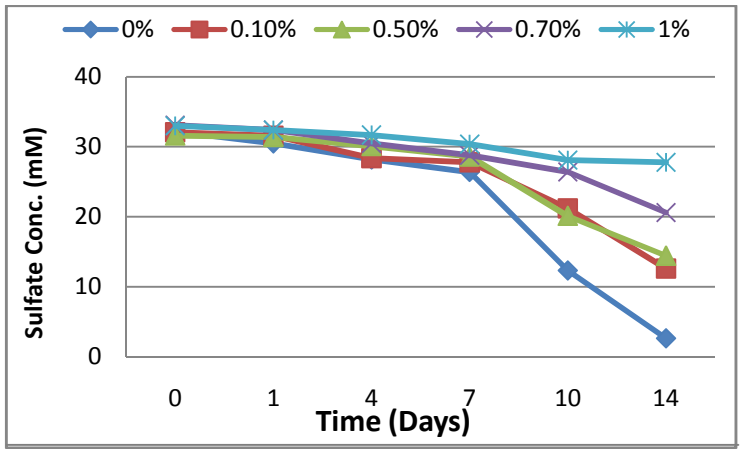


2d. So-NRB (Sulfide)

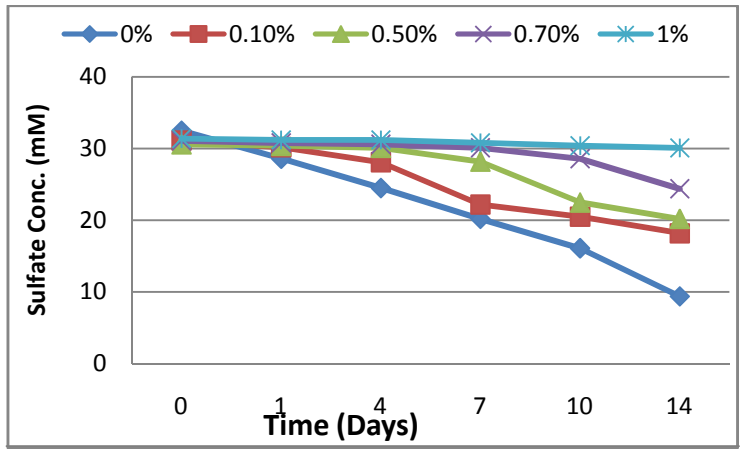


2e. So-NRB (Nitrate)

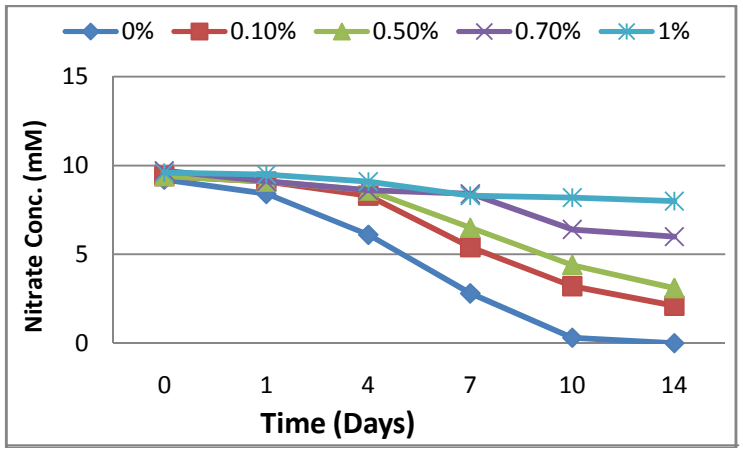
Fig. 2. Microbial activities in untreated produced water sample incubated with various concentrations of sodium azide for 14 days



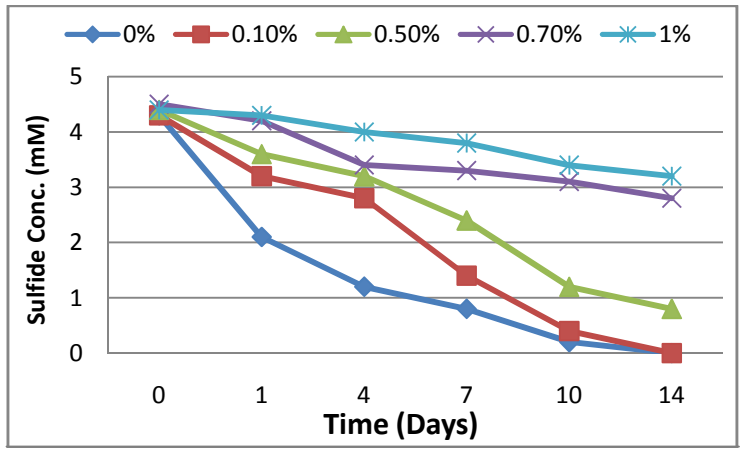
3a. SRB_LS (Sulfate)



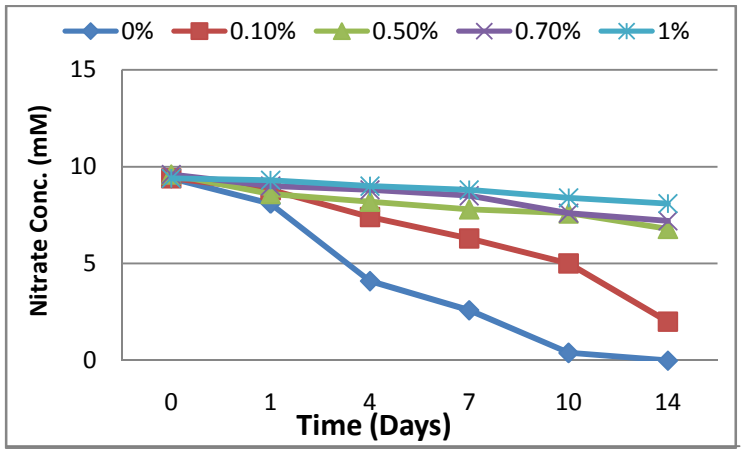
3b. SRB_VS (Sulfate)



3c. hNRB (Nitrate)

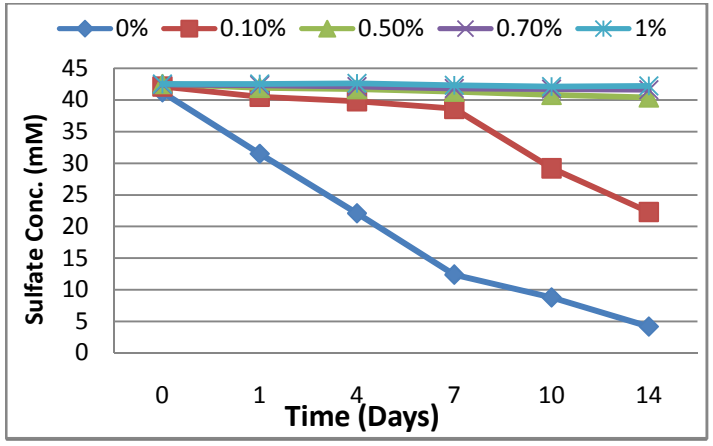


3d. so-NRB (Sulfide)

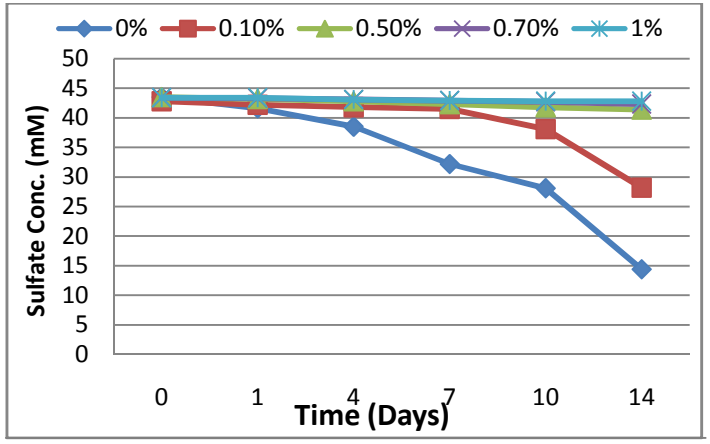


3e. So-NRB (Nitrate)

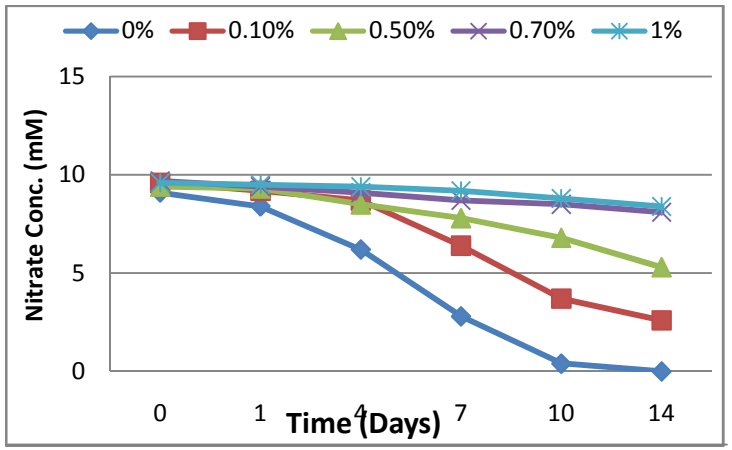
Fig. 3. Microbial activities in untreated produced water sample incubated with various concentrations of isothiazolone for 14 days



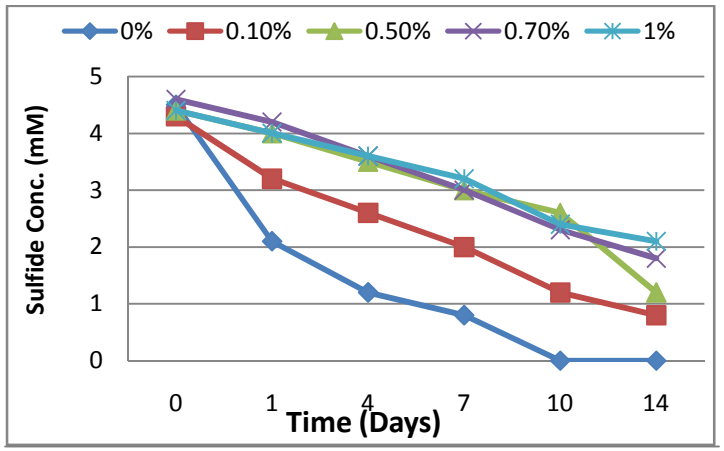
4a. SRB_LS (Sulfate)



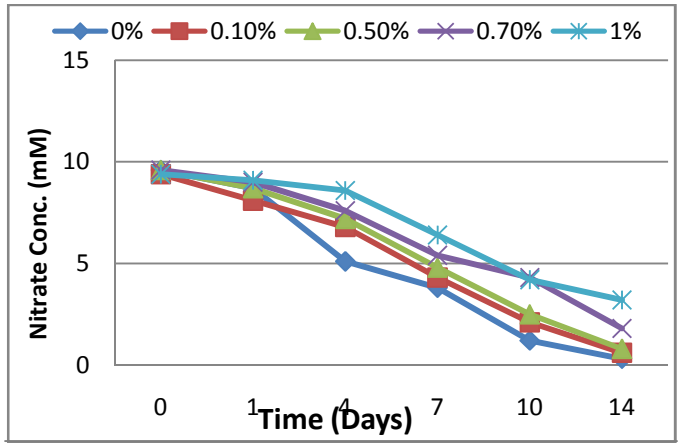
4b. SRB_VS (Sulfate)



4c. hNRB (Nitrate)

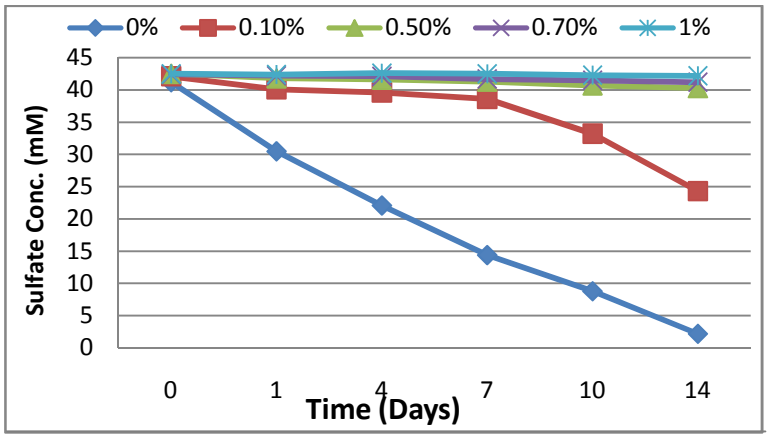


4d. so-NRB (Sulfide)

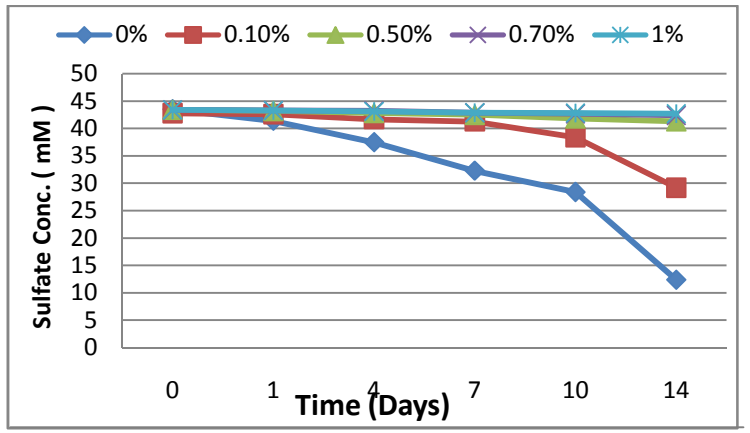


4e. so-NRB (Nitrate)

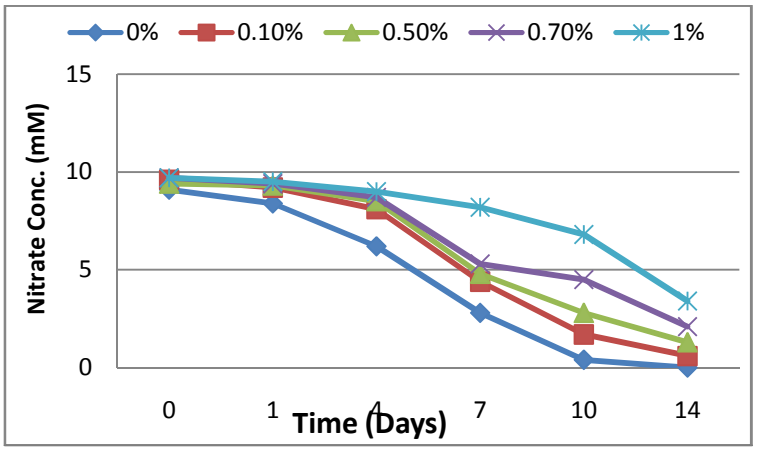
Fig. 4. Microbial activities in untreated injection water incubated with various concentrations of gluteraldehyde for 14 days



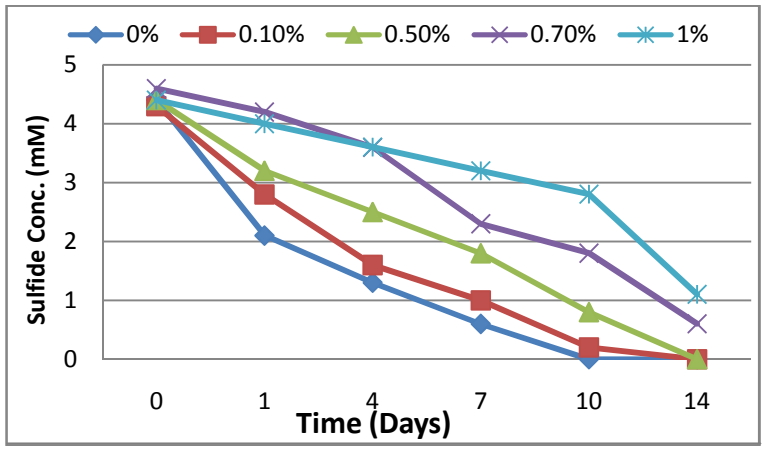
5a. SRB_LS (Sulfate)



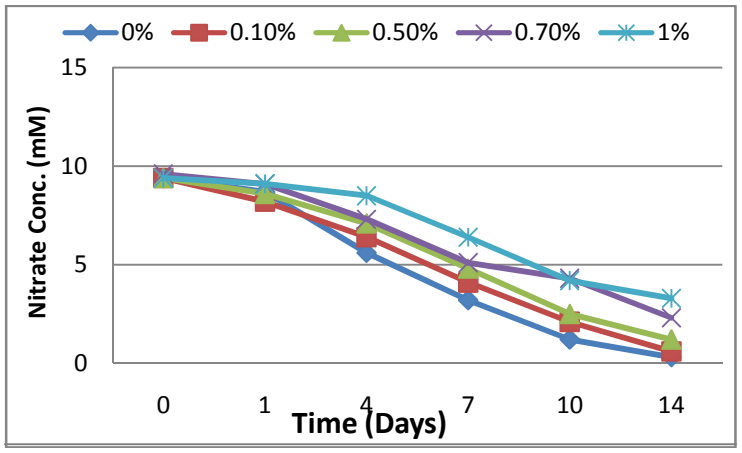
5b. SRB_VS (Sulfate)



5c. hNRB (Nitrate)

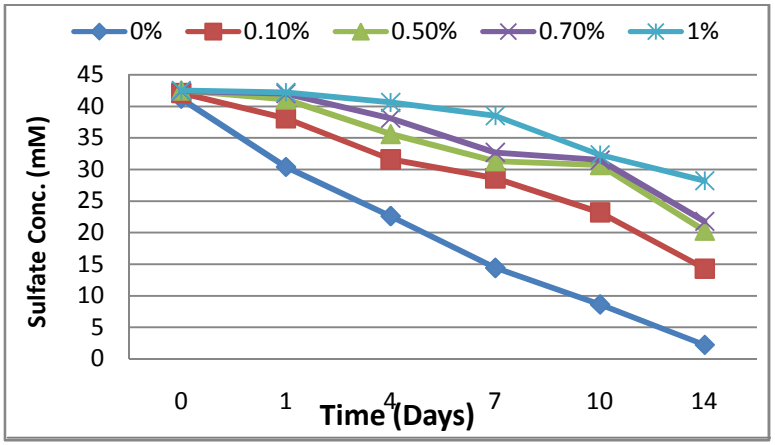


5d. so-NRB (Sulfide)

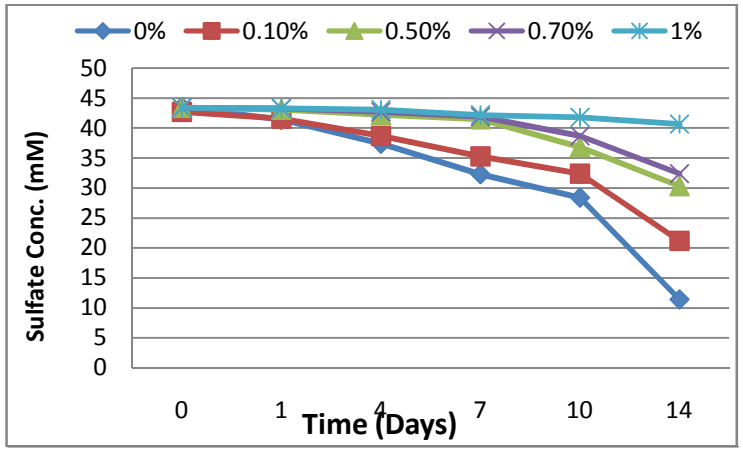


5e. so-NRB (Nitrate)

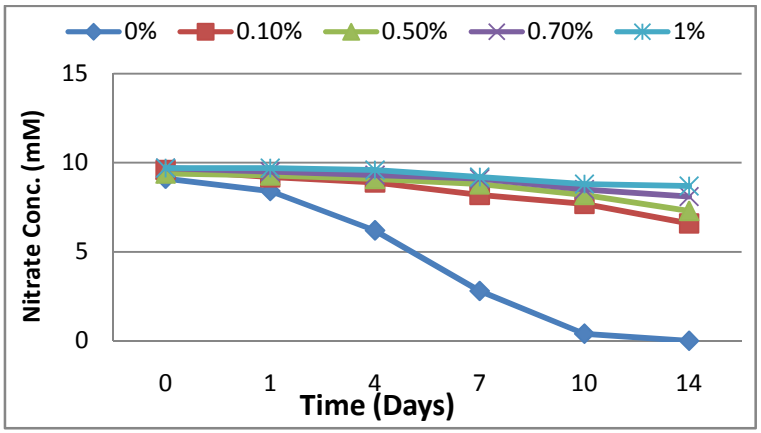
Fig. 5. Microbial activities in untreated injection water incubated with various concentrations of sodium azide for 14 days



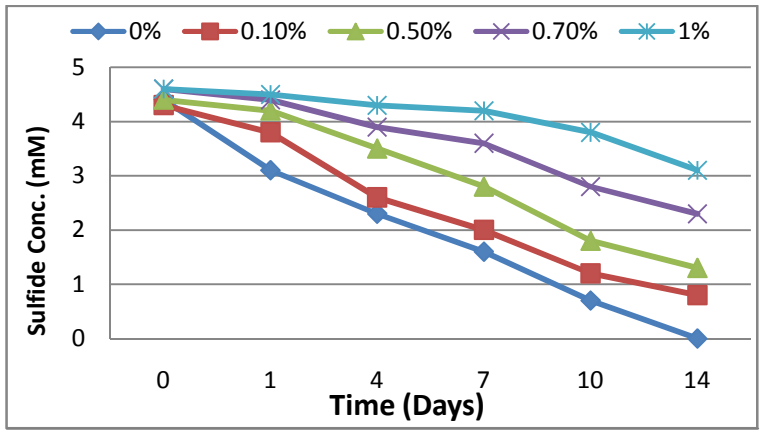
6a. SRB_LS (Sulfate)



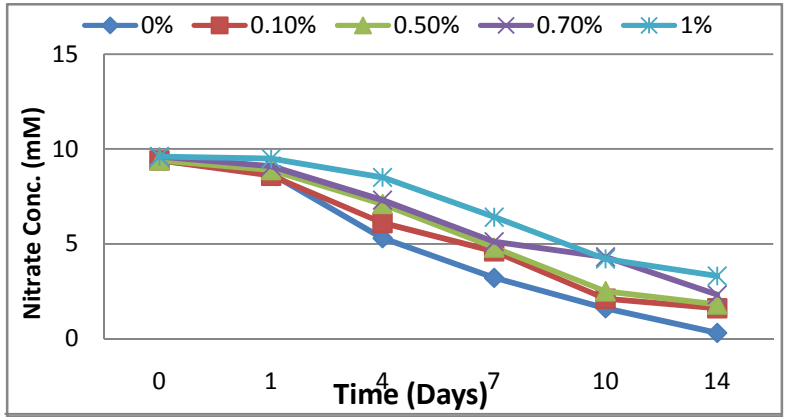
6b. SRB_VS (Sulfate)



6c. hNRB (Nitrate)



6d. so-NRB(Sulfide)



6e. so-NRB (Nitrate)

Fig. 6. Microbial activities in untreated injection water sample incubated with various concentrations of isothazolone for 14 days

3. DISCUSSION

MPN results showed that glutaradehyde at a lower concentration (0.5%), inhibited considerably both SRB and APB populations while sodium azide and isothiazolone recorded considerable inhibition of SRB and APB at a higher concentration (1%). On its effect on the functional group activities of microorganisms, glutaradehyde inhibited considerably at lower concentrations (0.5, 0.7%) the ability of SRB to reduce sulfate and generate sulfide by over 78% in both produced and injection water samples. Higher inhibition was however observed in injection water. Comparatively, the activities of hNRB and the so-NRB at higher concentrations of glutaraldehyde (1%) were affected by less than 30%. Further investigation is required to advance the positive selective action of glutaradehydes on the functional group activities of microorganisms. Glutaradehyde is a widely used biocide in oil fields because of its broad spectrum nature and biodegradability [6] but its effectiveness can be affected by acidic pH [16]. Gardner LR et al. [17] reported that 50ppm of glutaraldehyde retarded the SRB planktonic growth in Postgate C medium. Another report by Wen J et al. [6] indicated that 30 ppm of glutaradehyde combined with 2000 ppm of EDDS is effective in controlling planktonic SRB growth but for pre-grown SRB biofilm, a concentration of glutaradehyde as high as 500 ppm might not be effective. According to Greene EA et al. [18], the efficiency of glutaradehyde can be improved if it works in synergy with nitrites.

Sodium azide inhibited considerably the ability of SRB to reduce sulfate and generate sulfide by over 75% in both lactate and VFA media. Inhibition rate was however higher in injection water than in produced water. The activities of the hNRB and the so-NRB were also affected. Unlike glutaraldehyde, sodium azide did not exhibit a selective action. Sodium azide is a known efficient and strong biocide and lower corrosion rates have been observed in pipelines treated with sodium azide biocides [9].

Isothiazolones also showed some low level inhibition of SRB, hNRB and so-NRB activities when compared with the other two biocides (Glutaradehyde and Sodium azide). Williams TM et al. [10] advanced that Isothiazolones uses a two-step mechanism involving rapid inhibition of growth and metabolism followed by irreversible cell damage resulting in loss of viability and cell death. According to [5] Turkiewecz A et al. Isothiazolones are used only in an alkaline medium and at pH>7, they lose their biocidal properties. This explains why isothiazolones are not considerably inhibitory to acid producing bacteria and other microbial groups that generate acid during metabolism as shown in the present study.

Presently, no biocide works alone and efficiently in the industry. They are either used in combination with other biocides or with some biocide enhancers and chelators such as EDTA and EDDS, methanol and D-amino acid can also be added to improve efficiency [19,20]

A clear understanding of the activities of the three main microbial functional groups described in the present study can lead to the development of an efficient biocide with a selective action against problem causing microbes. At very low concentrations, the proposed biocide can work in synergy with beneficial microbes to completely eliminate the problem-causing ones. 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. 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 NR-SOB (so-NRB) 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 [21]. 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 [21]. 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 [18,21]. Naturally, so-NRB and hNRB are very useful for the control of souring and corrosion and need not be inhibited by biocides.

4. CONCLUSION

The present study has demonstrated how a clear understanding of the activities of the three main microbial functional groups can lead to the development of an efficient biocide that can selectively inhibit problem-causing microorganisms while working in synergy with beneficial ones. Further research is still required to further prove the positive selective action of glutaradehyde as demonstrated in this study and how the biocide can work in synergy at low concentrations with beneficial microorganisms to completely inhibit the activities of the SRB and other problem causing microorganisms. This will likely eliminate the persistent problems of microbial resistance to biocides in the oil and gas industry.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Sanders PF. Novel Methods for controlling microbial problems without using bactericides. Saudi Aramco J Technol. Summer. 2003;2-14.
2. Sunde E, Thorstenson T, Torsvik T. Growth of bacteria on water injection additives. 65th conf. SPE. ATCE. New Orleans. Los Angeles. USA. 1990;727.
3. Huang R T. Microbial influenced corrosion in cargo oil tanks. Presentation to the NACE 7-14B Marine Vessel Corrosion Committee. Houston TX. NACE International; 1996.
4. Sadip C, Carlton DH, Pamela JR, Amy LS, Corey LW. Evaluation of biocides for potential treatment of blast water. Final report to US coast guard research and development center. 1082 shennecossett Rd. CT. 06340-6048. Project no. 2004;4125.
5. Turkiewecz A, Brzeszcz J Kapusta P. The application of biocides in the oil and gas industry. NAFTA-GAZ Journal. 2013; 49:103-111.
6. Wen J, Zhao T, Gu T, Raad I. A green biocide enhancer for the treatment of sulfate reducing bacteria (SRB) biofilms on carbon steel surfaces using Glutaraldehyde. Intern Biod Biodet. 2009;63:1102-1106.
7. Klechka EW. Use of corrosion inhibitors on the trans Alaska Pipeline. Supplement to material performances. Cortec Corp. Alaska (pub). 2000;23.
8. Hayat MA. Chemical fixation. In: Hayat MA (ed). Principles and Techniques of Electron Microscopy: Biological applications. 4th ed. Cambridge University Press, Cambridge UK. 2000;28-45.
9. Okoro CC, Amund OO, Samuel OB. Biologically active solid deposits in biocide treated oil and gas pipelines from a Nigerian onshore oil production facility. International J Ecol Environ Sci. 2013;39(1):51-58.

10. Williams TM. The mechanism of action of Isothiazolone biocides. Power Plant Chem. 2007;9(1):16-22.
11. Morton LH, Greenway DA, Gaylarde CC, Surman SB. Consideration of some implication of biofilms to biocides. International Biodet Biod. 1998;41:247-259.
12. Stoodley P, Dodds I, Boyle J, Lappin-Scott H M. Influence of hydrodynamics and nutrients on biofilm structure. J Appl Microbiol Symp Suppl.1999;85:19-23.
13. Fux CA, Consterton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends in Microbiol. 2005;13:34-40.
14. Eaton AD, Clesceri LS, Greenberg A E. Standard Methods for the Examination of Water and Wastewater (19th edition). United books press (Pub.). Batimore MD. 1995;1126.
15. Truper HG, Schlegel HG. Sulfur metabolism in Thiorhodaceae. Quantitative measurements in growing cells of *Chromatium okehii*. Antonie van leewenhoek.1964;30:225-238.
16. Al-Hashem A, Carew J, Al-Borno A. Screening tests for fix dual biocide regimes against planktonic and sessile populations of bacteria. Paper presented at the 9th Iranian Chemical Engineering congress. Iran University of Science and Technology, Tehran. 23-25, Nov; 2004.
17. Gardner LR, Stewart PS. Action of glutaraldehyde and nitrate against sulfate reducing bacteria biofilms. J Ind Microbiol Biotechnol. 2002;29:354-360.
18. Greene EA, Brunelle V, Jenneman GE, Voordouw G. Synergistic inhibition of microbial sulfide production by combinations of the metabolic inhibitor Nitrite and Biocides. Appl Environ Microbiol. 2006;72(12):7897-7901.
19. Hubert C, Voordouw G. Oil field reservoir souring control by nitrate reducing *Sulfurospirillum spp*. that out compete sulfate reducing bacteria for organic electron donors. Appl Environ Microbiol. 2007;73(8):2644-2652.
20. Xu D, Wen J, Gu T, Raad I. Biocidal cocktail consisting of Glutaraldehyde, Ethylene Diamine Disuccinate (EDDS) and Methanol for the mitigation of souring and bio-corrosion. Corosion. 2012;68:994-1002.
21. Voordouw G. Emerging oil field biotechnologies. Prevention of oil field souring by nitrate injection. In Bioenergy. Wall et al. (eds). ASM press, Washington DC. (pub). 2008;379-388.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=546&id=8&aid=4945