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Potency and Implications of Bacteria Growth, H_2S and FeS Production in Microbially Induced Corrosion of Oil Pipelines using Selected Biocides

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Abstract
In this study, the potency and implications of bacteria growth, H_2S and FeS production in microbially induced corrosion of oil pipelines were done using 5 biocides. Hydrogenase test was carried out and used to detect the presence of micro-organism that causes corrosion in the pipeline samples. The biocides were then compared to ascertain how efficiently they can curb the proliferation of bacteria in experimental set-up filled with a bacterial cultured agar using anaerobic medium. Generally, it was found that there use led to reduction in bacteria growth, less and less of iron sulfide and hydrogen sulfide as the concentration and duration of experimentation increases. Specifically, it was found that while cow urine and biaguanide terminate their iron sulfide production after 5th and 6th week, respectively, others continue throughout the 13 week experimentation period. Similarly, cow urine and biaguanide terminate their hydrogen sulfide production after 3rd and 5th, respectively, others continued throughout. Altogether, 10 exponential trendline equations were formulated for iron sulfide and hydrogen sulfide production. R^2 goodness-of-fit statistical technique was employed in the analysis for future predictions from the model and the values obtained in each case is close to 1 which indicates a good measure that future outcomes are very likely to be predicted well by the developed equations.
Keywords: Biocides, Microbial Corrosion, Hydrogen Sulphide, Iron Sulphide, Bacteria Growth

1. Introduction

The chemical reaction that takes place in the corrosion process is reduction-oxidation (redox) reaction, which allows a specie of the material that is oxidized (the metal) to lose an electron at the anodic region while oxidizing agent gains an electron and becomes reduced and it takes place at the cathodic region (Wolfang 2002). During the corrosion process, the chemical, physical and the mechanical properties are all altered and hence the reason behind corrosion manifesting essentially as either uniform corrosion or localized attack. The later is less visible, less predictable, no definite control because it is localized in which an intense attack takes place only in and around a particular zone of the metal without affecting the remaining parts, this is the case of the microbially induced corrosion (Hardy and Brown,1986). Microbially induced corrosion is a major issue for the petroleum industries. It is not fundamentally different from any other type of electrochemical corrosion. It is simply that the chemical or physical condition giving rise to the environment is produced by the metabolic bye-product of micro-organisms. Generally, it is the corrosion resulting from the presence and the activities of micro-organisms including bacterial and fungi.

The roles of micro-organisms in the corrosion of metals have been the subject of numerous articles. It is therefore an interdisciplinary subject that embraces the field of mechanical engineering, chemistry, microbiology and biochemistry. All cases of microbially induced corrosion on external surface of pipes are associated with disbanded coatings and emphasize on the complexity and diversity of the metabolic process (Pope and Morris, 1995). It has been established that depolarization of cathode by hydrogen utilizing bacteria such as sulphate reducing bacteria is the mechanism of this type of corrosion, this results in localized corrosion in the form of pits and for some bacteria, the production of hydrogen sulphate deposits (Little, et al,1996). Similarly, It should be noted that micro-organisms often excretes surfactants leading to fuel emulsifications, and thus the probability of microbial penetration into a hydrophobic phase of the fuel increases (Waires et al, 2001). An anoxic condition combined with high numbers of sulfate reducing bacteria (SRB) in oil pipelines, installations and oil reservoirs result in the production of H_2S (Vance and Thrasher, 2005). H_2S is a toxic and corrosive gas that is responsible for a variety of environmental hazards and economic losses, including those due to microbially induced corrosion (MIC), reservoir souring and the consequently low production of oil in places (Davidova et al., 2001; Eckford and Fedorak, 2002). The rate of pitting corrosion has been attributed to sulfate and thiosulfate reducing bacteria (Crolet, 2005).

Research has shown that around 20-30% of corrosion deposits are related to the activities of micro-organism. The activities of these organisms affect the integrity, safety and reliability of petroleum pipeline operations. The organism causes problems from production strings to transmission pipelines and through refinery, storage and even in end user vehicle fuel tanks and fuel filters. This pipeline corrosion causing-organism slows down production, causes product quality loss, health hazards in polluted areas through spillage and a great cost burden to oil industries (Chung et al.,2000). Typically, the metabolic product of a growing microbiological colony accelerates the corrosion process by interacting with the corrosion products to prevent natural film-forming characteristics of the corrosion products that would inhibit further corrosion and by providing an additional reduction reaction that accelerates the corrosion process. Recent research has shown that in determining the influence of organism on pipeline corrosion, concentration should be based on the measures of the activities of the bacteria rather than their absolute numbers. Other organism responsible for the propagation of MIC includes metal oxidizing bacteria (MOB), acid producing bacterial (APB) and metal reducing bacteria (MRB) (Chung et al,2000).

2 Materials and Methods

2.1 Hydrogenase Test

For the purpose of this study, the hydrogenase kit test was used to detect the presence of micro-organism causing corrosion in the sample. Samples of oil and water mixture, corrosion products were collected from buried oil pipeline during maintenance known as Nembe-Brass trunkline belonging to the Italian oil giant Agip (ENI) which is an export terminal in the coastal town of Brass in Bayelsa State, Nigeria. Samples were collected in 650ml sterilized anaerobic serum bottles and were taken to a laboratory for further analysis. Samples of water were filtered to concentrate cells and the sludge was used without pre-heating. The sample was placed in a vial containing an enzyme – extracting solution. After 15 minutes contact, the solution was filtered and placed on a clean vial in an anaerobic chamber. A gas generator was activated and the oxygen in the chamber was removed by reaction with the hydrogen generated. The enzyme then oxidizes the excess hydrogen and simultaneously reduces the indicator dye in the solution. Hydrogenase test is a reliable indicator of the activities of corrosion-causing organism and it can be performed on water or deposit samples.

The urine of a female indigenous jersey cow was used in this study. It was collected from animal husbandry in Agbor. The cow was physically healthy as at the time the urine was collected and the detailed history of the cow was obtained from the owner regarding the age, milk yield, feeding practice and previous illness. The urine sample was collected using a urine collection container cup that is leak-resistant and amber colored to protect light sensitive analyses in the urine as recommended by NCCLS guidelines.

2.2 Bacteria Culture Using Anaerobic Medium

The second experiment carried out was the bacterial culture using anaerobic medium. The agar used for the bacteria culture test was an anaerobic medium which contains per litre of distilled water 0.5g K_2HPO_4, 2g yeast extract, 0.3g KH_2PO_4, 0.1g KCl, 0.2g $CaCl_2$. $2H_2O$, 0.5g NH_4Cl, 0.5g $MgCl_2.6H_2O$, 30g NaCl, 0.7g HCl, 0.5mg resazurin, and the medium was boiled under a stream of oxygen free nitrogen gas and cooled to room temperature and the pH is 7.4. The mixture (oil and water) was separated from the sample by keeping it at 40^0C for 60 minutes and allowing the water to settle at the bottom of the serum bottle. The pH of the water was adjusted by the addition of 1mol/L of NaOH and was read on a pH scale to give 7.

The medium was dispensed into six serum bottles each having a capacity of 45ml, the medium was inoculated with the water samples. The inoculated serum bottles was kept at 30^0C, which was constantly checked weekly, the incubation period lasted for 21 days before a visible change could be noticed.

2.3 Laboratory Test for Controlling MIC

The Anaerobic experimental pipes was used for this study. Steel coupons used for the experiment is rectangular AMS mild steel coupons with dimension (1.27cmx7.62cmx0.16cm) and the weight is 49.60g. These coupons were positioned inside downstream of the experimental pipes. Five experimental anaerobic pipes were used and each was filled with prepared anaerobic medium that contained the cultured bacteria. Before the beginning of the experiment, the experimental pipes were washed with detergents, rinsed with distilled water and autoclaved. The coupons were degreased with ethanol and rinsed with acetone before insertion; the pipes were run in a circulation mode for one day (1 day) under anaerobic condition and then inoculated. Five biocides were chosen which include Formaldehyde, Diamines, Polyamines, Biguanides and Cow Urine. Biocides were injected in the five anaerobic pipes ranging from the concentration 5mg/l to 580mg/l. In all the treatment given at weekly intervals, the mixture was circulated in a closed loop at 30^0C for 13 weeks representing the standard duration used in the treatment of microbial induced corrosion within the petroleum industries. The turbidity and the blackening were observed weekly in the five anaerobic pipes having the different biocides of varied concentration and the coupons in each of the pipe weighed. Similarly, the bacteria growth, hydrogen sulfide(H_2S) production, Iron (II) production (Fe(II))

3 Results and Discussions

3.1 Hydrogenase Result

After 3 hours contact of the solution in the anaerobic chamber in the presence of activated gas generator, the development of a blue color was noticed, this confirmed the activities of hydrogenase by the micro-organism. The intensity of the color is proportional to the rate of hydrogen uptake by hydrogenase enzyme, the test is a reliable indicator of the activities of corrosion causing organism. It will have particular application in the analysis of sample from the biofilms probes, and this biofilms probes can be introduced directly into the reaction vials for enzyme extraction. The presence of hydrogenase enzyme on the metal surface has been found to be a reliable indicator of the potential microbial corrosion.

3.2 Bacterial Culture Result

After the incubation period of 21days, the development of black color becomes visible and also a repulsive smell like that of a rotten egg was equally noticed. The image of the inoculated medium shows the presence of black film which confirms the presence of iron sulfide (FeS) precipitate in conjunction with the presence of hydrogen sulfide that has a repulsive smell like that of a rotten egg. The observations above gave rise to the conclusion that sulphate reducing bacterial (SRB) are responsible for the corrosion.

3.3 Determining the Bacteria Growth against Biocides

From the experiments carried out for 13 weeks, the bacteria growth measured in (cfu/ml) for each of the biocide is shown in Table 1 and a log scale was used to effectively show the results in Fig 1

Table 1: Bacteria Growth of the Biocides for various Concentrations/Week

Week	Concen-tration (mg/l)	Bacteria Growth (cfu/ml) of the Biocides				
		Formal-dehyde	**Polyamine**	**Diamine**	**Cow Urine**	**Biaguanide**
1st	5	10^6	10^5	10^6	10^3	10^2
2nd	30	10^5	10^5	10^5	10^2	10^2
3rd	80	10^4	10^5	10^5	10^2	10^2
4th	130	10^4	10^3	10^5	-	10^2
5th	180	10^4	10^3	10^4	-	10^2
6th	230	10^3	10^3	10^4	-	10^2
7th	280	10^3	10^3	10^4	-	-
8th	330	10^3	10^3	10^3	-	-
9th	380	10^2	10^3	10^3	-	-
10th	430	10^2	10^2	10^3	-	-
11th	480	10^2	10^2	10^3	-	-
12th	530	10^2	10^2	10^2	-	-
13th	580	10^2	10^2	10^2	-	-

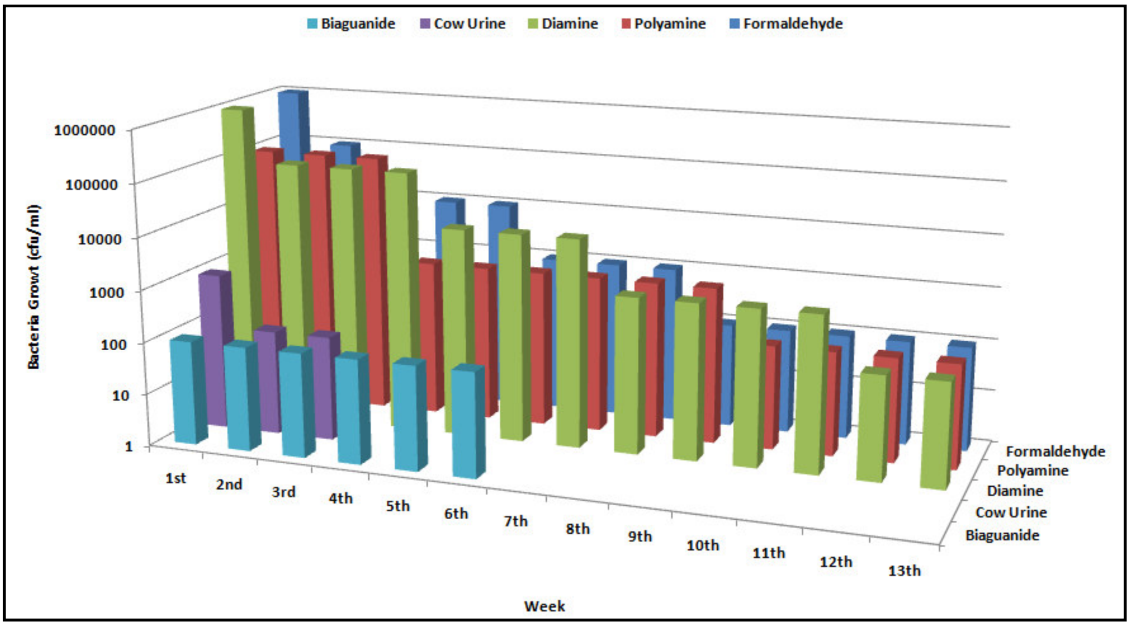

Fig 1: Bacteria Growth of the Biocides for various Concentrations/Week

It can be observed generally that the bacteria growth reduces as the concentration and experimental duration increases. On a specific note, biaguanide shows no bacteria growth above the 6th week and concentration of 230mg/l while cow urine gave the best result by not showing any bacteria growth beyond the 3rd week and 80mg/l concentration. The result above shows the effectiveness of cow urine in killing as well as inhibiting bacteria growth.

3.4 Hydrogen Sulfide Production of the Biocides

The results of the hydrogen sulfide (H_2S) production of each of the five biocides at varying concentrations for the 13 weeks duration are tabulated in Table 2 and the chart is plotted in Fig. 2

Table 2: H_2S Production of the Biocides for various Concentrations/ Weeks

Week	Concen-tration	H_2S Production (Mmol/L) of the Biocides				
		Formal-dehyde	Polyamine	Diamine	Cow Urine	Biaguanide
1st	5	4.4	4.8	4.01	4.4	4.7
2nd	30	4.1	4.3	3.73	0.71	3.28
3rd	80	3.6	3.2	3.71	0.22	1.09
4th	130	2.4	2.5	2.66		0.09
5th	180	1.3	1.42	2.01		0.05
6th	230	0.9	0.99	1.65		
7th	280	0.88	0.83	1.64		
8th	330	0.78	0.79	1.29		
9th	380	0.59	0.62	1.06		
10th	430	0.53	0.34	0.84		
11th	480	0.44	0.33	0.75		
12th	530	0.32	0.32	0.74		
13th	580	0.28	0.21	0.72		

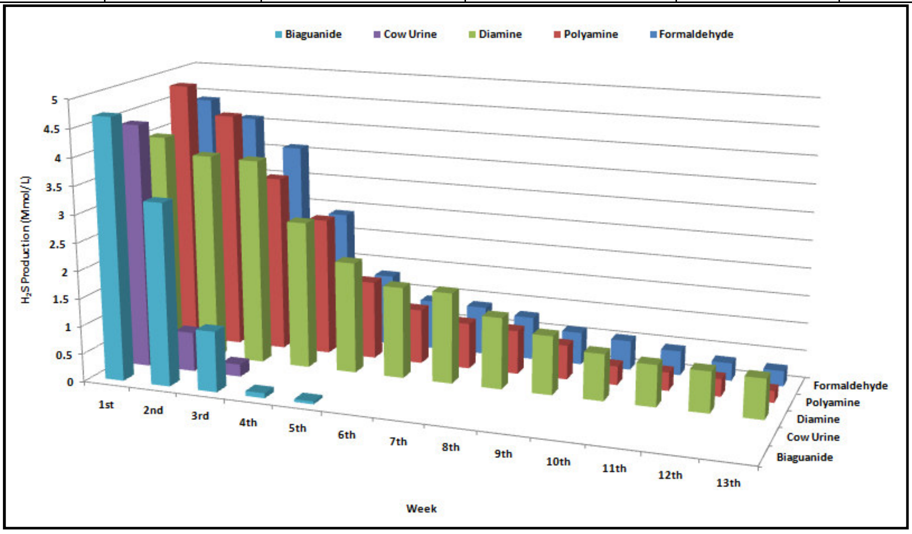

Fig 2: H_2S Production of the Biocides for various Concentrations/Weeks

Table 3: Values of R, R^2 and Trendline Equations of Biocides for H_2S Production

Biocides	R	R^2	Trendline Equations for F_2S Production
Formaldehyde	0.981	0.963	$y = 5.603e^{-0.24x}$
Polyamine	0.988	0.977	$y = 6.345e^{-0.16x}$
Diamine	0.985	0.971	$y = 4.950e^{-0.16x}$
Cow Urine	0.992	0.984	$y = 17.65e^{-1.49x}$
Biaguanide	0.965	0.932	$y = 26.79e^{-1.26x}$

On a general note, it can be observed from Table 2 and Fig. 2 that the biocides produce less and less of hydrogen sulfide as the concentration and week increases. On a specific note however, while formaldehyde, polyamine and diamine produced hydrogen sulfide throughout the 13-week duration of the experiment, cow urine and biguanide do not. The cow urine terminates the hydrogen sulfide production after the 3rdweek and a concentration of 80mg/l while the biguanide stops its hydrogen sulfide production after the 5th week and 180mg/l concentration. The implication of the result is that the less the hydrogen sulphide is produced, the less the iron sulphide will be deposited. Hence, from the biocides considered, the cow urine appears as the best biocide out of the rest for microbial corrosion under consideration.

Table 3 shows the values of the coefficient of determination (R^2) and the trendline equations for H_2S Production of the five biocides. The R^2 values for each of the biocides were close to 1 which indicates a good measure that future outcomes are likely to be predicted well by the trendlines. Specifically, from the trendline equations, the degree of correlation for formaldehyde, polyamine, diamine, cow urine and biaguanide are 98.1%, 98.8%, 98.5%, 99.2% and 96.5%. This means that the models results can come out true to reality up to the above percentages when applied.

3.5Iron Sulfide Production of the Biocides

The results of the FeS production of each of the five biocides at varying concentrations for the 13 weeks duration are tabulated in Table 4 and the chart is plotted in Fig. 4

Table 4: FeS Production of the Biocides for various Concentrations/ Weeks

Week	Concen-tration	FeS Production (Mmol/L) of the Biocides				
		Formal-dehyde	Polyamine	Diamine	Cow Urine	Biaguanide
1st	5	18.6	16	19	10	16
2nd	30	15.4	12	19	4.6	12
3rd	80	12.4	12	18.2	3.05	8
4th	130	8.4	10.9	16	0.52	4.3
5th	180	8	8.7	8.2	0.02	2.3
6th	230	8	5.7	8		1.08
7th	280	7.78	5.1	7.8		
8th	330	7.78	4.7	7.2		
9th	380	7.48	4.7	7.2		
10th	430	7.4	2.2	7.2		
11th	480	4.2	1.4	6.1		
12th	530	3.2	1.4	5		
13th	580	2.4	1.2	4.6		

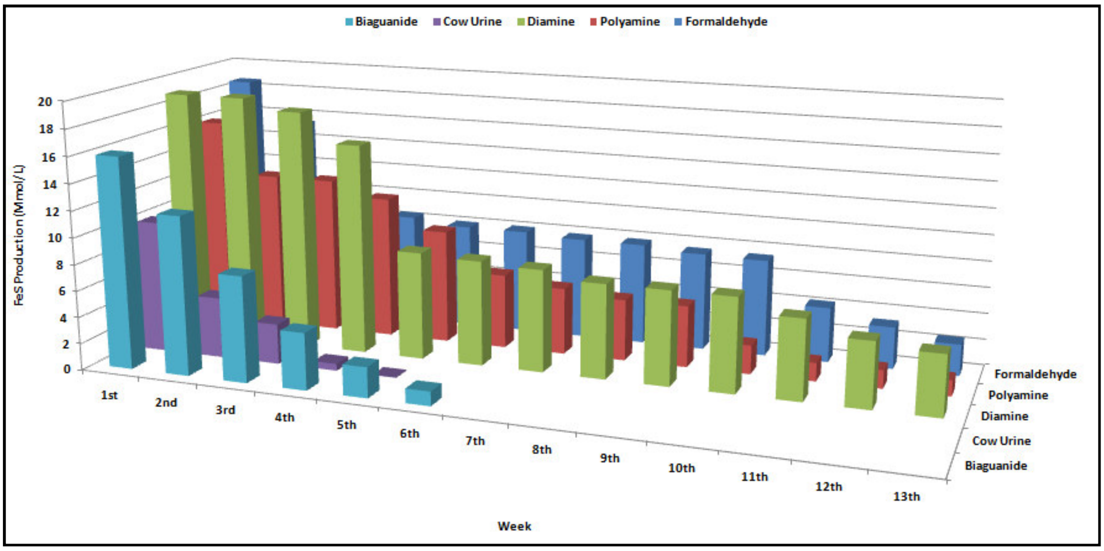

Fig 4: FeS Production of the Biocides for various Concentrations/Weeks

Table 5: Values of R, R^2 and Models of Biocides for FeS Production

Biocide	R	R^2	Trendline FeS Production
Formaldehyde	0.930	0.864	$y = 19.41e^{-0.13x}$
Polyamine	0.975	0.951	$y = 23.34e^{-0.22x}$
Diamine	0.942	0.887	$y = 21.4e^{-0.22x}$
Cow Urine	0.932	0.868	$y = 86.34e^{-1.46x}$
Biaguanide	0.988	0.976	$y = 33.88e^{-0.54x}$

It is easily observed from Table 4 and Fig. 4 that the biocides produce less and less iron sulfide as the concentration and week increases. On a specific note however, while formaldehyde, polyamine and diamine produced hydrogen sulfide throughout the 13-week duration of the experiment, cow urine and biguanide do not. The cow urine terminates the iron sulfide production after the 5th week and a concentration of 180mg/l while the biguanide stops its iron sulfide production after the 6th week and 230mg/l concentration. The implication of the result is the confirmation that the less the hydrogen sulphide is produced, the less the iron sulphide is deposited. Hence, from the biocides considered, the cow urine appears as the most potent biocide out of the rest for microbial corrosion.

Table 4 shows the values of the correlation coefficient (R), coefficient of determination (R^2) and the trendline equations for FeS Production of the five biocides. The R^2 values for each of the biocides were close to 1 which indicates a good measure that future outcomes are likely to be predicted well by the developed model trendlines. Specifically, from the trendline equations, the degree of correlation for formaldehyde, polyamine, diamine, cow urine and biaguanide are 93.0%, 97.5%, 94.2%, 93.2% and 98.8%. This means that the equations results can come out true to reality up to the above percentages when applied.

4.Conclusion

This study has established the potency of using 5 biocides (i.e. formaldehyde, diamines, polyamines, biguanides and cow urine) to curb microbial induced corrosion in oil transmission pipelines. Bacteria growth, H_2S and FeS production reduces with the use of the biocides as experimental duration increases. However, some biocides are more efficient than others. Specifically, it was found that cow urine and biaguanide terminate their iron sulfide production after 5th and 6th week, respectively. Similarly, cow urine and biaguanide terminate their hydrogen sulfide production after 3rd and 5th, respectively. The implication of the study is that both cow urine and biaguanide stop the growth of microbial solution as witnessed in this study which will consequently stop the propagation of microbial induced corrosion when used in oil transmission pipelines.

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