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Original Research Article

Biocidal Effects of Ozone, Sodium Hypochlorite and Formaldehyde, on Sulphate Reducing Bacteria Isolated from Biofilms of Corroded Oil Pipelines in the Niger Delta, Nigeria

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The performance of three biocides (ozone, sodium hypochlorite and formaldehyde) on Sulphate reducing bacteria was tested. The Sulphate reducing bacteria were isolated from biofilms of corroded oil pipelines under normal flow condition. The study was aimed at controlling these bacteria using the three biocides at different concentrations (0, 1, 2, 3, 4 and 5 percent) in order to reduce the economic losses normally encountered in the oil and gas industry due to the activities of Sulphate reducing bacteria. The results showed that ozone was effective from 1% concentration by completely eliminating *Desulfuromonas acetoxidans, Desulfobulbus propionicus* and *Desulfosarcina variabilis* followed by sodium hypochlorite and then formaldehyde being the least effective. For proper monitoring and control of microbiologically influenced corrosion caused by sulphate reducing bacteria, Ozone and one of the non-oxidizing biocides such as formaldehyde should be used.

Keywords: Biocides, Sulphate Reducing Bacteria, Biofilms, Corroded Oil Pipeline, Economic Losses.

INTRODUCTION

Corrosion resulting from the attachment and activities of microorganisms on metal surfaces is referred to as microbiologically influenced corrosion (MIC) or biocorrosion (Videla, 1996). It occurs in diverse environments and is not limited to aqueous submerged conditions, but also takes place in humid atmospheres. It is an electrochemical process in which the participation of microorganisms is able to initiate, facilitate or accelerate the corrosion reactions without altering the process electrochemical nature.

Corrosion is one of the main damages causing severe economic losses in pipeline systems of the petroleum industry. About 40% of the internal pipeline corrosion in the gas industry has been attributed to microbiologically influenced corrosion (Graves and Sullivans, 1996). In pipelines, biocorrosion takes place when microbial consortia interact with metallic surfaces through a cooperative global metabolism (Frenchel, 2002). Microbiologically influenced corrosion occurs virtually in all industries, including paper and pulp, sugar, denting, shipping, gas and petroleum industries [Bermont Bouis *et al.,* 2007). Generally, the major bacteria involved in the corrosion of petroleum production systems are the anaerobic sulphate reducing bacteria (SRB), especially under anoxic conditions(Beech and Sunner 2004). Sulphate reducing bacteria are able to couple organic matter oxidation with the reduction of sulphite, sulphate or thiosulphate. This metabolism leads to the production of sulphides which react with metallic surfaces (Widdel Lihle et al., 2000).

Usually, biocorrosion caused by SRB or any other microorganisms can be controlled by the application of biocides and corrosion inhibitors. These biocides can be either oxidizing or non-oxidizing biocides. Some examples of oxidizing biocides of industrial use include the following: chlorine, ozone, and bromine (Videla and Herrena 2005). The non-oxidizing biocides are more effective in the overall control of algae, fungi and bacteria, because they are more persistent and many are pH dependent Videla Herrena and (2005). Typical non-oxidizing biocides are formaldehyde, glutaraldehyde, isothiazalones and quaternary ammonia

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compounds. Increasing legislative requirements and the necessity for greater environmental acceptability have contributed to restricting the use of some common biocides and to developing either new compounds or carefully selected blends of existing biocides, taking into consideration the use of ozone for different industrial system present several advantages as compared to other biocides (Rice and Wilk (1991)

The unique combination of the toxicity of ozone during treatment makes ozone the biocide of choice for the present decade provided an appropriate balance between positive effects and costs is reached (Stritt matter et al. (1992). In this study, we used ozone, sodium hypochlorite and formaldehyde to test on the sulphate reducing bacteria.

MATERIALS AND METHODS

The Study area

The Niger Delta is located within latitudes $5^{o}45'$ and $6^{o}35'$ and longitudes $4^{o}50'$ and $5^{o}15'$ in the central parts of Southern Nigeria and has a land mass covering 70,000 square kilometers which accounts for about 8 percent of Nigeria's land mass (NDES, 2003).

The ecology of the area is characterized by a vast flood plain build up by the accumulation of sedimentary deposits washed down into the Niger and Benue Rivers. The area is criss-crossed with numerous rivers, streams, tributaries, creeks and creeklets. The vegetation of the area is characterized by (1) sandy coasts ridge barriers, brackish or saline mangrove forest (2) fresh water swamp forest (3) tropical rainforest. The area is the largest wetland in West Africa and one of the largest mangrove forests in the world (Darafeka 1998).

The meteorological study of the area reveals an average atmospheric temperature of $25.5^{o}C$ in the rainy season, and $30^{o}C$ in the dry season. The daily relative humidity values ranged from 55.5 percent in the dry season to 96 percent in the rainy season (Gobo 1998). The area is characterized by two distinct seasons, wet and the dry seasons. The wet season starts from March to October, while the dry season starts form November to March. A short dry spell is normally noticed in August and it is traditionally referred to as "August Break". The rainfall ranges from 2000-3000 mm per annum. The Niger Delta is where petroleum industries are sited in Nigeria with distribution of onshore and offshore oil fields.

It is estimated that Nigeria earns over 90 percent of its foreign exchange and over 80 percent of government revenues from the oil industries (Okoko and Nna 1998).

Characteristics of biocides used

Ozone, it is a bluish gas with a characteristic pungent odour. Ozone is an oxidizing biocide that is partially soluble in water and highly unstable as it readily reverts to oxygen. The solubility of ozone in water dependent on the amount of ozone in the carrier gas stream. Thus, it is important to produce a gas stream containing a relatively high amount of ozone. For instance, The maximum solubilities at $25^{o}C$ for gas stream containing 1 and 3% ozone are 2.7 and 8.1ppm, respectively (Rice and Wilkes, 1991) These maximum levels are not obtained in practice because of out-gassing of the carrier gas which removes some of the dissolved ozone. Solubility of ozone also decreases with increasing temperature. Ozone degrades with pH, being stable under certain condition of pH 6 and stable at pH 10 (Rice and Wilkes, 1991).

Sodium hypochlorite (NaOCl)

Is a pale yellow liquid. In its pure form, sodium hypochlorite is unstable and reacts with water to form hypochlorous acid (NOCl) and sodium hydroxide (NaOH). At the pH values ranging from 7 to 9, hypochlorite acid can be converted to hydrogen ion (H^{+}) which is an effective biocide and hypochlorite anion (OCl^{-}). At the pH 7.5, about equal amount of hydrogen ion and hypochlorite anion are present. At the pH of 8.5, the split is about 10% hydrogen ion and 90% hypochlorite anion (Aieta *el al*., 1980).

Formaldehyde (HCHO)

Is a non-oxidizing biocide. It is a liquid that is readily soluble in water because of the presence of polar carbonyl group. It is an effective biocide that acts both on protein and lipopolysaccharides of the bacterial cell envelope. It is known to be effective even at low concentration of 0.1ppm (Longely *et al*., 1980).

Sampling of Biofilms

To obtain the biofilms, ten mild steel coupons (surface area $35.2cm^2$ and density $7.57g/cm^3$ each) obtained from a commercial source (metal samples company, Munford Al). The coupons used have the same chemical compositions as the mild steel pipelines with the following chemical composition: 0.06% e, 1.05% Mn, 0.27% Si, 0.06% P, 0.002% S, 0.02% Cr, 0.02% Ni, 0.08% Mo, 0.05% V, 0.02% Ti, 0.05% Al, 0.02% Cu and 98.424% Fe, using metal samples corrosion monitoring systems (serial No 16021 of metal sampling company, Munford, Al), coupons were inserted into the inner surface of the pipelines (with 9", 10", 14", 18" and 24" diameters) through the access valves for a period of 127 days (Fig 1).

At the end of the 127 days, the coupons were detached from the inner regions of the pipelines and the biofilms formed were scraped with sterile razor blades and collected into sterile bottles containing 5 ml phosphate buffered saline at pH 7.0 according to (Sambrook et al., 1989). The bottles were stored in a cooler of ice block and at the end of the day transferred to the laboratory for analysis. Each coupon was named after the pipeline in which it was inserted, these were: OSH 01, OSH 04, OSH 13, OSH 17 and EOC 04 in Oshie Flow Station (Fig 2).

Culture Medium/Determination of Sulphate Reducing Bacteria

The medium of choice was Postgate B medium containing the following compositions: KH_2PO_4 0.5g/l, NH_4Cl 1.0g/l, $CaCl_2.6H_2O$ 0.1g/l, $MgSO_4.7H_2O$ 2.g/l, sodium lactate (60-70%) 5 ml i.e yeast extract 1.0g/l, Ascorbic acid 0.1 g/l, thioglycolic acid 0.1g/l, $FeSO_4.7H_2O$ 0.5 g/l, NaCl 26 g/l distilled water 100 ml and the pH adjusted to 7.0. The medium was prepared according to (Postgate, 1984). Briefly, 37.3g of the medium were suspended into 100 ml of distilled water and sterilized by autoclaving at $121^{o}C$ for 15 minutes and allowed to cool before dispensed into sterile Petri dishes.

0.1ml aliquot of serially diluted biofilm samples was dispensed on to Petri dishes in triplicates using spread plate technique. The inoculated plates were stored in an anaerobic jar and incubated for a period of seven days.

Fig. 1. Biofilms on coupons

Fig. 2. Collection of biofilms

At the temperature of 37^0C At the end of the seven days incubation, the plates were removed and the black colonies formed were counted as sulphate reducing bacteria and the mean expressed as cfu/ml.

Maintenance of Pure Culture

Discrete colonies were purified by repeated subculture unto pastgate B medium. Pure cultures were preserved on postgate B slant and stored in the refrigerator at 4^oC for further tests. Characterization and identification of microbial isolates. Pure cultures of SRB isolates were identified based on cultural parameters, miscrosapic techniques and biochemical tests including carbohydrate utilization and hydrogen sulphide production. Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of known taxa as in (Bergey and Breed, 1957).

Determination of effect of each Biocide on the SRB

To determine the effect of each biocide on the SRB, the tube dilution technique was employed according to (Madigan et al., 2009). Briefly postgate B broth was suspended into 1000 ml of distilled water and heated gently to dissolve completely. The medium was then dispensed into test tubes with the name of each isolate on it and according to the percent biocide to be used, for example, 1% biocide for 99ml broth, 2% biocide for 98ml broth, 3% biocide for 97ml broth, 4% biocide for 98ml broth, 5% biocide for 95ml broth and sterilized by autoclaving at 121^oC for 15 minutes.

After cooling, the biocides were added to the test tubes and the tests tubes then inoculated with each isolate. The control test tubes were also inoculated with the isolates but with no biocide added. The tubes were packed into and anaerobic jar and incubated for 5 days at 37^oC. At the end of the incubation period, the tubes were tested for turbidity to determine the minimum inhibitory concentration (mic) of each biocidee against the organisms colorimetrically at 680nm.

Preparation of Biocides

Ozone

- 1% ozone was prepared by adding 1 ml of the ozone to 99ml of Postage B broth
- 2% was prepared by adding 2 ml of the ozone to 98ml Postage B broth.
- 3% was prepared by adding 3 ml of the ozone to 97ml Postage B broth.
- 4% was prepared by adding 4 ml of the ozone to 98ml Postage B broth.
- 5% was prepared by adding 5 ml of the ozone to 95ml Postage B broth.

Sodium hypochlorite and f**ormaldehyde** were prepared in the same manner.

RESULTS AND DISCUSSION

The biochemical characteristics of sulphate reducing bacteria isolated from biofilms of corroded oil pipelines in Rivers State revealed the occurrence of three sulphate reducing bacteria. The species of sulphate reducing bacteria detected in the biofilms included the following: *Desulfuromonas acetoxidans, Desulfobulbus propionicus* and *Desulfosarcina variabilis*. Due to the economic losses as well as environmental health and safety hazards caused by the activities of communities of mixed sulphate reducing bacteria in many industrial sector such as the oil and gas industries, it is necessary to check the risks resulting from the activity of these organisms (Ghazy et al., 2011).

The growth frequency and the activity of these organisms caused severe corrosion problems in the oil and gas pipelines. The activity of these microorganisms can be minimized or completely eradicated by the application of antimicrobial agents or biocides. One of the effective ways to measure the effect of biocides on an organisms is by determining minimum inhibitory concentration (mic), which prevent growth in a suitable medium.

In this study the effects of three biocides (ozone, sodium hypochlorite and formaldehyde) at different concentrations (0, 1,2,3,4 and 5 percent) on the three sulphate reducing bacteria isolated from biofilm of corroded oil pipelines were tested. The results in Fig. 3-5 showed the effect of ozone, sodium hypochlorite and formaldehyde on the three sulphate reducing bacteria, while Figs 6-8 showed the efficiency of each biocide against the sulphate reducing bacteria.

The result in Fig. 3 shows that the three biocides decreased *Desulfuromonas acetoxidans,* and the degree of depression is with increasing concentration. At one percent concentration ozone had completely wiped out *Desulfuromonas acetoxidans* from the medium. The next biocide reducing *Desulfuromonas acetoxidans* was sodium hypochlorite followed by formaldehyde.

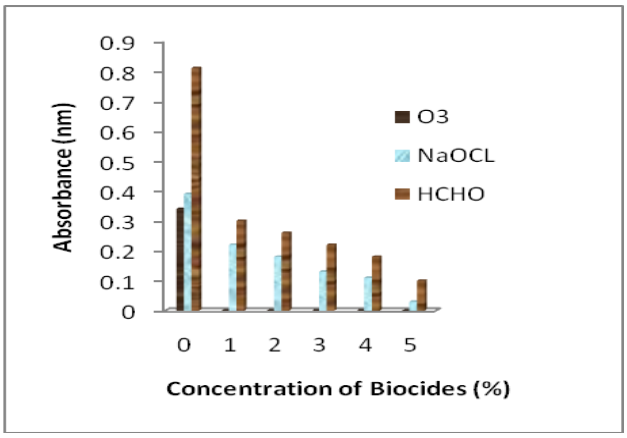

Fig. 3. Effects of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfuromonas acetoxidans*

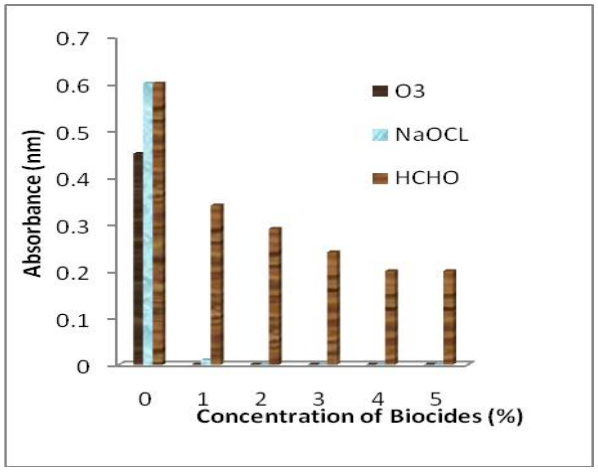

Fig. 4. Effects of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfobulbus propionicus*

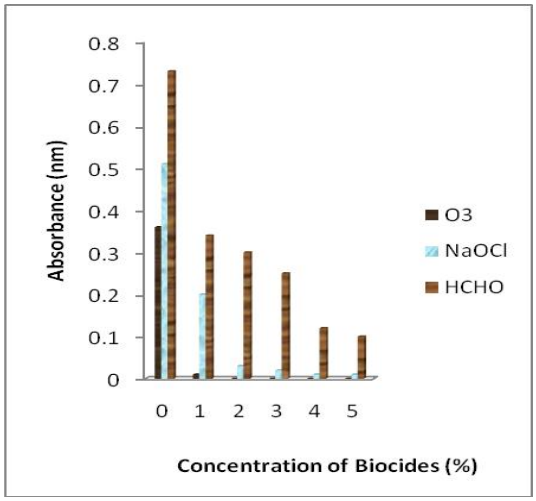

Fig. 5. Effects of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfosarcina variabili*

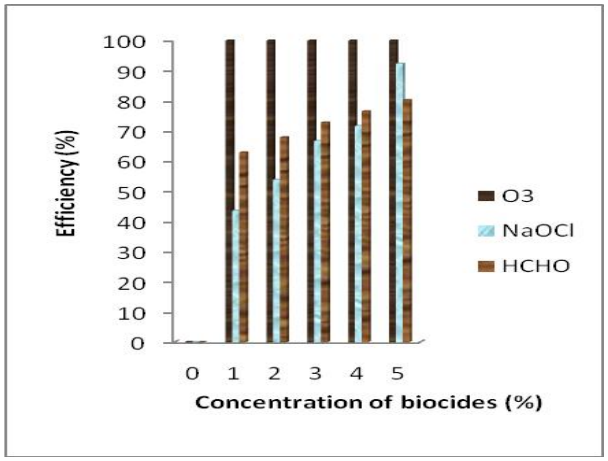

Fig. 6. Efficiency of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfuromonas acetoxidans*

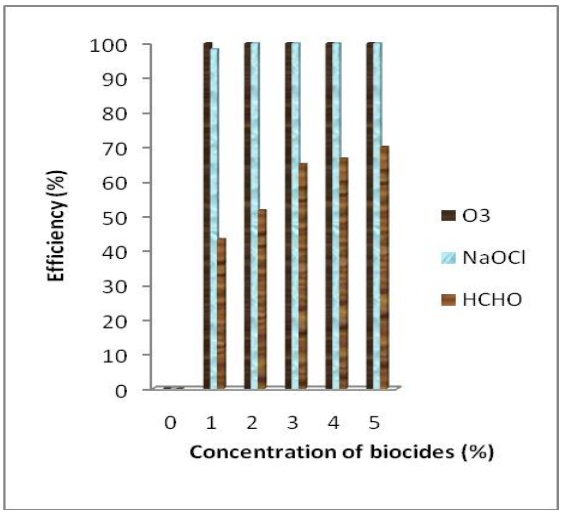

Fig. 7. Efficiency of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfobulbus propionicus*

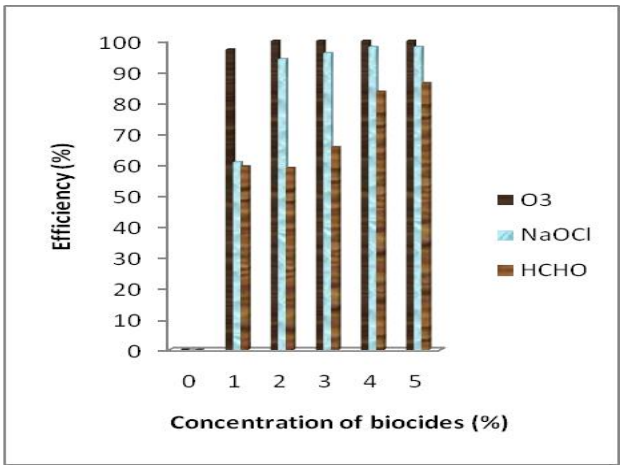

Fig. 8. Efficiency of Ozone, Sodium hypochlorite and Formaldehyde on *Desulfosarcina variabilis*

The result here may probably be that *Desulfuromonas acetoxidans* is capable of degrading sodium hypochlorite and formaldehyde and use them as sources of nutrients for the development of it protoplasm and therefore needed higher concentrations for complete reduction.

The result in Fig. 4 shows the effect of three biocides on *Desulfobulbus propionicus* the result followed the same trend. Indicating depression of the organism with increasing concentration level of the biocides with ozone wiping off completely, *Desulfobulbus propionicus* at one percent concentration, followed by sodium hypochlorite wiping off the organism at two percent concentration level, and then formaldehyde being the least effective. The result in Fig. 5 shows the effect of the three biocides on *Desulfosarcina variabilis*. The result showed reduction of *Desulfosarcina variabilis* by the three biocides.

The degree of depression of *Desulfosarcina* increased as the concentration of each biocide increases, and this effect was more pronounced with the ozone followed by sodium hypochlorite and then formaldehyde. For instance, Fig. 5 indicated that ozone wiped out *Desulfosarcina variabilis* from one percent concentration followed by sodium hypochlorite and then formaldehyde being the least. This results confirmed earlier report by Videla and Herrena (2005) that ozone is very effective against bacteria. Puyate and Rim –Rukeh 2008) also reported that ozone at the contraction of 0.1 and 0.2 will eliminate microorganisms in produced water.

Fig. 6 shows the performance of each biocide against *Desulfuromonas acetoxidans.* The result showed that ozone exhibits the best biocidal efficiency against *Desulfuromonas acetoxidans* with 100 percent efficiency. This is followed by formaldehyde with percent efficiency ranging from 62.92 – 80.24 percent, and then sodium hypochlorite with percent efficiency ranging from 43.58-92.30 percent. This indicated that *Desulfuromonas acetoxidans* is capable of resisting sodium hypochlorite and formaldehyde or may be the bacterium is capable of degrading the biocides at lower concentrations, using them as substrates for protoplasm development.

Fig. 7 showed the efficiency of the three biocides against *Desulfobulbus propionicus.* The result shows ozone and sodium hypochlorite exhibiting the best biocidal efficiency against *Desulfobulbus propionicus* with 100 percent efficiency respectively, followed by formaldehyde showing the least efficiency with the percent efficiency ranging from 43.33 to 70 percent. This result indicates that *Desulfobulbus propionicus* is capable of resisting formaldehyde up to five percent level of concentration. It therefore means that *Desulfobulbus propionicus* needed higher concentrations before it can be wiped out completely from the pipelines. The efficiency of the three biocides against *Desulfosarcina variabilis* is as shown in Fig. 8. The result indicates that ozone and sodium hypochlorite exhibited the best biocidal efficiency against *Desulfosarcina variabilis* with 100 percent efficiency respectively, followed by formaldehyde with 59.42 to 86.30 percent efficiency.

Fig. 3 - 6 showed the performance of the three biocides, at the same concentration of the biocides, ozone exhibited the best biocidal efficiency followed by sodium hypochlorite and then formaldehyde as the least effective of the biocides. Biocide is said to be effective if it can achieve at least 4 log reductions of total planktonic microorganisms (Costerton 1984; Gaylarde 1992). This is exhibited by the three biocides; more especially ozone even at lower concentration eliminated all the three sulphate reducing bacteria followed by sodium hypochlorite and formaldehyde being the least.

CONCLUSION

The effect of three biocides (ozone, sodium hypochlorite and formaldehyde) at different concentrations (0, 1, 2, 3, 4 and 5 percent) were test tested on sulphate reducing bacteria isolated from biofilms of corroded oil pipelines. The results showed that the three biocides depressed all the three sulphate reducing bacteria with increasing level of concentration with ozone being the best efficient followed by sodium hypochlorite, and then formaldehyde being the least effective. This indicates that to check the infestation for sulphate reducing bacteria in oil pipelines, Ozone and Sodium hypochlorite should be adequately applied.

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