Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.19, 2013

Effects of Different Concentrations of Biocides on Fungal Populations Isolated from Biofilms of Corroded Oil Pipelines

Godwin U. Akpan[1] and Gregory S. Effiong[1], Boniface D. Akpan[2]
[1]Department of Soil Science and Land Resources Management,
University of Uyo, P.M.B. 1017, Uyo, Nigeria
[2]Department of Animal Science, University of Uyo, P.M.B. 1017, Uyo, Nigeria
E-mail of the corresponding author: godwinuakpan@uniuyo.edu.ng, agumoren1@yahoo.com

ABSTRACT
Microbiologically influenced corrosion is a problem commonly encountered in facilities in the oil and gas industries. The present study described fungal enumeration and identification in biofilms of oil pipelines in Oshie flow station in Rivers State and Irri flow station in Delta State, Nigeria using traditional cultivation technique. The fungal species isolated in biofilms from the two sites are as follows *Verticillium dahlae, Saccharomyces cerevisiae, Penicillium corylophilum, Botrytis cinerea, Fusarium paradoxus, Aspergillus paradoxus, Humicola grisae, Aureobasidium pullulans, Monilia balanitis, Hormoconis resinea, Asperillus flavus and Helimenthosporium maydis*. The study also focuses on the use of three biocides to inhibit or eliminate the identified organisms in order to minimize the material and financial losses encountered by oil and gas companies, because of microbiologically influenced corrosion (MIC). The performance of three biocides (ozone, sodium hypochlorite and formaldehyde) at the concentrations 0, 1, 2, 3, 4 and 5% in eliminating the fungal species isolated from biofilms of oil pipelines in Rivers and Delta States, Niger Delta, Nigeria. It is shown that formaldehyde an ozone exhibit the best biocidal characteristics and concentrations of 1 and 2 % eliminated all to almost the fungal species after 72 hours of contact time. This study is relevant to the problem of microbiologically influenced corrosion as the data may contribute to elucidate which fungal species contribute to the MIC process and to gain a better understanding of the fungal community of biofilms. This study will give us better understanding of the biocide capable of eliminating fungal species in biofilm of oil and gas pipelines.
Key word: Concentration, fungal population, biocides, microbiologically influenced corrosion, biofilms

1. Introduction

Microbiological influenced corrosion of mild steel pipelines is an important economical problem facing oil and gas industries. Microbes form tubercles, which block fluid flow and can facilitate localized corrosion leading to through-wall penetrations. Microbes of diverse physiological types and metabolism potentialities have been recovered from fresh tubercles or under-deposits corrosion and characterized. Microbiological influenced corrosion is increasingly recognized as a serious problem when metal surfaces are exposed to natural waters (Pope *et al.*, 1984).

Microbiologically influenced corrosion or biocorrosion is a corrosion problem in the oil and gas industries facilities. These may occur when microbial consortia interact with metallic surfaces through the establishment of multispecies biofilms in which different microorganisms contribute to corrosion through a co-operative global metabolism (Little and Ray 2002; Frenchel 2002). Corrosion affects the operation and maintenance cost of the pipelines, and many oil pipelines face severe corrosion and biofouling problems (Benka Coker *et al.* 1995).

Generally, most microbiologically influenced corrosion studies focused only a bacterial involvement, however, under aerobic conditions, other singled celled organisms such as fungi, yeast and diatoms can also influenced corrosion (Prasad, 2000).

The predominant types of fungi associated with MIC are acid producing fungi (*Hormoconis resiaea*) hydrocarbon utilizing fungi(Penicillium sp.and Candida species), heterotrophic fungi (*Saccharomycess sp*) and fungi secreting organic acids and slime (Puyate and Rim-Rukeh, 2008).

These organisms coexist within a biofilm matrix of metal surfaces, functioning as a consortium in a complex and coordinated manner. The various mechanisms of biocorrosion reflex the variety of physiological activities carried out by these different types of microorganisms when they coexist in biofilms (Al-Saleh *et al.*, 2011). The activities of these microorganisms causing corrosion of petroleum pipelines may be controlled or inhibited by the application of biocides or surfactants (Lechevallier *et al.*, 1988). Examples of biocides are chlorine, sodium azide, glutaraldehyde and sodium hypochlorite (Videla, 1996).

Despite decades of study on MIC, it is still not known with certainty how many species of microorganisms contribute to corrosion and researchers continue to report on the formation of biofilms by an ever-widening range of microbial species. However, in this study, we have isolated and identified different fungal species from biofilms of oil pipelines. This study was therefore designed to identify other microorganisms

other than heterotrophic and sulphate-reducing bacteria involved in the corrosion of oil pipelines and the control measures in order to eliminate the losses faced by oil and gas industries.

2. Materials and Methods

2.1 Sampling of Biofilms

To obtain the biofilm samples, twenty mild steel coupons (surface area 4.57cm^2, each and density 7.57 g/cm^3 each) were placed at the inner surface of each pipeline (Fig. 1). The coupons have the same chemical composition as the pipelines. Two coupons were placed per pipeline and exposed to the flow of petroleum for a period of 127 days. The involved pipelines were located at the Oshie flow station in Rivers State and Irri flow station in Delta State of Nigeria. Five pipelines were marked for sampling per flow station; overall, ten pipelines were sampled. The coupons were detached from the inner regions of the oil pipelines and the biofilms, formed were removed with sterile razor blades and collected into sterile bottle with 10ml phosphate buffered saline pH 7.0 (Sambrook *et al.,* 2000).

The biofilm samples were name according to the involved pipelines in Rivers State are: OSH 01 (7TBG), OSH 04 (6LS), OSH 13 (6 SS), OSH 17 (11 SS), EOC 04 (OBF 31 LS), Pipelines marked for sampling in Delta State are: Irri 02 (Irri 4LS), Irri 06 (Irri 2T), Irri 07 (ISK 4LS), Kwale 05 (Kwale IPP), Kwale 06 (8LS),

2.2 Microbiological Analysis

i) **Serial Dilution**: Ten-fold serial dilutions of the biofilm samples were made as outlined by (Collins and Lyne) *Harrigan* and McCance 1976).

ii) **Inoculation and Incubation:** One milliliter of ten-fold serially diluted biofilms samples were inoculated onto Sabouraud Dextrose Agar plates in triplicates using pour plate technique (Collins and McCance 1976), inoculated plates were incubated at 28^{o}C for 48-72 hours for the enumeration of total heterotrophic fungi. Visible discrete colonies in incubated plates were counted and expressed as colony forming units per ml (cfu/ml) of biofilm samples.

(iii) Maintenance of pure culture discrete colonies was purified by repeated sub-culture unto Sabouraud Dextrose Agar. Pure culture were preserved on Sabouraud Dextrose Agar slants and stored in the refrigerator (4^{o}C) for further tests and identification (IV) characterization and identification of fungal isolates. Fungal isolates were identified based on the taxonomic schemes of (Lodder, 1974 and Domsch *et al.,* 1980). Briefly, the wet mount for examination and identification of fungal isolates was employed using lactophenol in cotton blue stain. A drop of lactophenol in cotton blue stain was placed on a clean slide, using sterile inoculating needle, a loopful of 5-7 days cultures were transferred unto clean grease free slides. The specimens were flooded with lactophenol in cotton stain for 3-5 minutes. The slides were carefully covered with cover slip to avoid air bubbles and then mounted on the microscope. The slides were then placed under a X 40 objective of the microscope to focus.

The following features were looked for and recorded: colony colour, types of stoma, nature of hypha, special vegetative structures, asexual spores, special reproductive structures, conidial head and vesicle shapes.

2.3 Preparation of Culture Medium/Biocide

The medium of choice for the determination of minimum inhibitory concentration is Sabouraud Dextrose Broth. Thirty grammes of Sabouraud Dextrose Broth was suspended in 1000 ml of distilled water and heated gently to dissolve the medium completely. The medium was dispensed into test tubes 99ml, 98ml, 97ml, 96ml, 95ml and 90ml for control, bearing the name of the organisms to be tested and the percentage biocides to be used (that is, 0,1,2,3,4,5%) then sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool before inoculated with the biocides and isolates.

2.4 Preparation of Biocides

i. Ozone

One per cent (1%) ozone was prepared adding 1ml ozone to the 99ml sabouraud dextrose broth (SDB).
Two per cent (2%) was prepared by adding 2 ml Ozone to the 98ml sabouraud dextrose broth.
Three per cent (3%) was prepared by adding 3ml Ozone to the 97ml sabouraud dextrose broth.
Four per cent (4%) was prepared by adding 4ml Ozone to the 96ml sabouraud dextrose broth and,
Five per cent (5%) was prepared by adding 5ml Ozone to the 95 ml sabouraud dextrose broth.

ii. **Sodium hypochlorite (NaOCl)**

One per cent (1%) was prepared by adding 1ml sodium hypochlorite to the 99ml sabouraud dextrose broth.
Two per cent (2%) was prepared by adding 2ml sodium hypochlorite to the 98ml sodium hypochlorite
Thee per cent (3%) was prepared by adding 3ml sodium hypochlorite to the 97 ml sabouraud dextrose broth.

Four per cent (4%) was prepared by adding 4ml sodium hypochlorite to the 96ml sabouraud dextrose broth, and 5% was prepared by adding Five per cent (5%) was prepared by adding 5 ml sodium hypochlorite to the 95ml sabouraud dextrose broth.

iii. **Formaldehyde (HCHO)**

One per cent (1%) was prepared by adding 1ml formaldehyde to the 99ml sabouraud dextrose broth.
Two per cent (2%) was prepared by adding 2ml formaldehyde to the 98ml sodium hypochlorite
Thee per cent (3%) was prepared by adding 3ml formaldehyde to the 97 ml sabouraud dextrose broth.
Four per cent (4%) was prepared by adding 4ml formaldehyde to the 96ml sabouraud dextrose broth, and 5% was prepared by adding Five per cent (5%) was prepared by adding 5 ml formaldehyde to the 95ml sabouraud dextrose broth.

2.5 Inoculation and Incubation of Isolates for MIC.

Antimicrobial activity is measured by determining the smallest amount of the agent needed to inhibit the growth of a test organism, a value called minimum inhibitory concentration (MIC). To determine the MIC for the three biocides (ozone, sodium hypochlorite and formaldehyde) against all the detected fungi, series of culture tubes were prepared and inoculated. Each tube contains Sabouraud Dextrose Broth with different concentration of the agents (0, control (with no biocide but distilled water), 2,3,4 and 5 percent). The tubes were incubated for a period of 48-72 hours at 28^{o}C. After incubation, the tubes were checked for visible growth (turbidity), and the MIC determined colorimetrically by measuring g the absorbance at 680nm (Table 1) control tubes were inoculated with sterile distilled water. The efficiencies (E%) of biocides (Table 2) were calculated according to the following equation:

$$E\% = \frac{E\ uninhibited - E\ inhibited}{E\ uninhibited} \times 100$$

3. Results and Discussion

The results in Figures 1 and 2 show the number of fungal species isolated from biofilms of oil pipelines in Rivers State and Delta State respectively. This indicated that twelve fungal species were isolated from biofilms of oil pipelines in both Rivers and Delta State.

These fungi included the following: *Verticillium dahlae, Saccharomyces cerevisiae, Penicillium corylophilum, Botrytis cinerea, Fusarium paradoxus, Aspergillus paradoxus, Humicola grisae, Aureobasidium pullulans, Monilia balanitis, Hormoconis resinea, Asperillus flavus and Helimenthosporium maydis,* one of the simplest ways to measure the effect of biocides on an organism is by determining the minimum inhibitory concentration (mic) which prevent growth in a suitable medium.

The results in Tables 1 and 2 showed the biocidal activity and biocidal efficiencies of each biocide at different concentration against each fungal specie isolated from biofilms of oil pipelines. It may be seen that ozone, completely eliminated Iso I (*Verticillium dahlae*) from one per cent with biocidal efficiency of 100% and then Formaldhyde with the biocidal activity of 0.01nm from 1 per cent level of concentration and the organism was completely wiped off from 2 per cent levels of concentration with the biocidal efficiency of 100 per cent. This was followed by sodium hypochlorite, which depressed the organism from 1% concentration level up to 4% concentration levels where the organism was eliminated with the biocidal efficiency ranging from 43.59-100%. The reason why ozone and formaldehyde have 100% efficiencies may be due to their ability to permeate the cell membrane of the microorganism.

This observation corroborate the reports by Bessems (1983), that the cell membrane of microorganisms is composed of several lipids and protein layers arranged together in a specific arrangement called the bilayer (or multi-layer lipoprotein structure). The presence of the lipids as a building unit in the cell membrane acquires them their hydrophobic character. The selective permeability of the lipoprotein membrane represents the main function, which control the biological reaction in the cell. Hence, any factor influencing that permeability causes a great damage to the microorganism, which leads to it dead. The biocidal activities and efficiencies of the three biocides against Iso 2 (*Saccharomyces cerevisiae*) shows that formaldehyde completely wiped out the organism from 2% levels of concentrations, with the biocidal efficiency of 71.42 in 1 per cent concentration and 100% from 2% to 5% concentration levels. This was followed by sodium hypochlorite and ozone that depressed the organisms and the degree of reduction of the organism increases as the concentration of each biocide increases. This indicates that after 72 hours of contact time 2% formaldehyde eliminated the organism followed by sodium hypochlorite and than ozone sodium hypochlorite and ozone did not achieve 100% efficiency on Iso 2, many be due to inability of the chemicals to permeate the cell. It may also be because Iso 2 is able to degrade the chemicals and use them as sources of carbon. This observation is in line with earlier report by Muthukumar et al., (2007), who opined that in the petroleum transporting pipelines, many biocides /some microorganisms, hence, degrade inhibitors used control measures, including the selection of biocides are important aspects for

petroleum industry. The biocidal activity and efficiency of the biocides against Iso 3 (*Penicillium corylophilum*) shows that formaldehyde and ozone completely eliminated the organism from 1 per cent and 2 per cent levels of concentrations respectively, after 72 hours contact time, followed by sodium hypochlorite which only depressed the organism and the degree of reduction of the organism increased as the concentration of the biocide increases. The reason why formaldehyde and ozone are able to completely wiped off the organism may be because the chemicals have the potency to kill the organism or inhibit growth and reproductive cycles of the organism by poisoning the microbial enzymes and causing protein denaturation, cell leakage and lysis. This observation corroborates the reports by Longley et al., (1980), who stressed that formaldehyde is an effective biocide that acts both on proteins and on lipopolysaccharides of organism's cell envelope. Formaldehyde is known to be effective even at low concentration of 0.1ppm; the same is true for ozone. Sodium hypochlorite did not completely wipe off the isolate even at 5% level concentration, because the biocide cannot permeate the lipoprotein membrane of the fungal cell. Although, the biocide did not completely wipe off the organism, but it has achieved up to 95.91 per cent efficiency, this is in line with the reports by Guiamet and de Saravia (2005), which showed that the effective concentration of biocide for the control of microbial growth in a system ranges from 0.1 to 0.5 ppm.

The biocidal activity and efficiency of the three biocides against Iso 4 and 5 (*Botrytis cinerae* and *Fusarium oxysporum*) (Tables 1 and 2) showed that the three biocides depressed the two isolates with increasing concentration level. However, Formaldehyde eliminated Iso 4 (*Botrytis cinerae*) and sodium hypochlorite eliminated Iso 5 (*Fusarium oxysporum*) from 1 per cent concentration level. Ozone only depressed Iso 4 and Iso 5 (*Botrytis cenerea and Fusarium oxysporum*) as the concentration of the biocides increases with efficiencies up to 97.82 per cent for Iso 4 and 5 respectively. This may be ascribed to the fact that the two organisms are able to degrade the chemicals and use them as sources of carbon. The effects of the three biocides against Iso 6 and 7 (*Aspergillus paradoxus* and *Humicola grisae*) showed that formaldehyde depressed Iso 6 and 7 as the concentration increases, and completely wiped off Iso 6 from 2 per cent concentration, whereas Iso 7 was completely eliminated from 4 per cent concentration. Ozone and sodium hypochlorite depressed the two organisms and the degree of reduction increased as the concentrations of the biocides increase, ozone is observed to wipe out Iso 6 from 4 percent concentration. The results reported here lend further evidence to the hypothesis that biocides are antimicrobial chemicals that have the potency to kill microorganisms or inhibit their growth and reproductive cycles by poisoning the microbial enzymes and causing protein denaturation, cell leakage and lysis.

The biocidal activity and efficiency of the three biocides against Iso 8 and 9 (*Aureobasidium pullulans* and *monillia balanitis* (Tables 1 and 2) depressed *Aureobasidium pullulans* and *Monilia balanitis* and the degree of reduction of the organisms increased as the concentration of the biocides increases. The biocide finally eliminated the two isolates at 3 per cent and 4, per cent respectively with 100 per cent efficiencies (Table 2) after 72 hours of contact time. Similarly, the same trend was observed in the case of sodium hypochlorite against the two organisms, except that the organisms were eliminated, but efficiencies of 97.92 per cent were achieved by the two biocides against the organisms.

The biocidal activity and efficiency of formaldehyde against Iso 8 and 9 showed that the biocide depressed *Aureobasidium pullulans* as the concentration increases with 97.78 per cent efficiency against the organism. On the other hand, the biocide depressed *Monilia balanitis* as the concentration increases with final elimination of the organism at 3 per cent concentration achieved the efficiency of 100 per cent against the organism. These results are in line with the report by Videla (1996) that biocide is said to be effective if it can achieve at least 4 log reduction of total planktonic microorganisms. In this study, the biocides used have achieved between 97.78-100 per cent efficiencies, indicating almost to total elimination of the organisms.

The biocidal activities and efficiencies of the three biocides against Iso 10 and 11 (*Hormoconis resinae* and *Aspergillus flavus*) (Tables 1 and 2) showed that the three biocides performed efficiently. It is observed that ozone depressed those two organisms with total elimination of Iso 10 at 3 per cent concentration and Iso 11 from 2 per cent concentration achieving efficiencies of 97.54-100 per cent respectively against, the organisms.

Similarly, the same result was observed on the effect of sodium hypochlorite against the organisms. It was also observed that formaldehyde showed the best biocidal efficacy against the two organisms by eliminating the organisms from 1 per cent concentration achieving 100 per cent efficiency against the organisms respectively. This result further confirms the report that formaldehyde is a non-oxidising biocide that acts both on proteins and on lipopolyscchasides of organism's cell envelope (Longley et al., 1980). The biocide is known to be effective even at low concentration of 0.1ppm. The results in Tables 1 and 2 show the performance of the same concentration of the three biocides on Iso 12 (*Helminthosporium maydis*).

It is observed that the three biocides performed effectively against the fungus by achieving 100 per cent, efficiencies respectively. As the concentration of a biocide increases, its performance also increases. This result also demonstrated that formaldehyde is known to be effective against microorganisms even at low concentration of 0.1 ppm (Longley, et al., 1980).

4. Conclusion

Laboratory investigations of the effects of different concentrations (0, 1, 2, 3, 4 and 5%) of each of the three biocides (ozone, sodium hypochlorite and formaldehyde) on 12 fungal species isolated from biofilms of corroded oil pipelines. It is shown that the performance of each biocide increases as the concentration increases at 72 hours contact time. The results further demonstrated that formaldehyde and ozone exhibited the best biocidal efficacy against all the fungal species, even up to 100 per cent efficiency against most of the fungi, followed by sodium hypochlorite. These three biocides have the potency to kill microorganism and inhibit their growth and reproductive cycles by poisoning the microbial enzymes and causing protein denaturation, cell leakage and lysis. The choice of these biocides is based on their reported environmental friendliness and stability over a wide range of pH. The present study aimed to detect and control fungal species inhabiting biofilms of oil pipeline in order to minimize the economic losses as well as environmental health and safty hazards caused by the activity of these organisms.

References

Al-saleh, M. A., Al-Ibrahim, T. M. T. Lundgaard, Sanders, PF; Sorensen K. B. & Jubler S. (2011). Microbiologically Influenced Corrosion (MIC) Assessment in Crude Oil Pipelines. Sandi Aramco Journal of Technology, Spring. 57-63.

Beech, I. B. & Gaylarde, C. C. Recent Advances in the Study of Biocorrosion. AD Overview, Rev. Microbiology. Vol. 30, No. 3, 1999, pp. 177 -190.

Benka-Coker M. O., Menseagharum W. & Ekundayo, J. A. (1995). Abundance of Sulphate reducing bacteria in Niger Delta oilfield waters. *Bioves tech.* 54: 151-154.

Bessems, E. (1983). "Biological aspects of the assessment of microbial corrosion" proceedings of the conference sponsored and organized jointly by the national physical laboratory and the metals society and held NPL. Teddintou on 8-11 March 1983. The metal society London.

Collin, O. H. & F. M. Lyne (1978). Microbiological methods. Great Britain: Butterworth and Company Limited.

Domsch, K. H. & H. Gams and T. H. Anderson (1980). Compendium of soil Fungi London: Academy Press.

Frenchel, T. 2002, Microbial Behaviour in Heterogeneous world. Science. 296: 1068-1071.

Guiamet, P. S. & S. G. G. de Saravia (2005). Laboratory studies of biocorrosion control using traditional and environmental friendly biocides. An overview, Latin Am. Applied res. 35: 295-300.

Harrigam, E. F. & M. E. McCame (1976). Laboratory methods in Food and Dairy Microbiology. London: Academic Press.

Lechevallier, M. W., Cawthous, C. D. & Lee, R. G. 1988. Inactivation of biofilms bacteria: *Appl. Environ. Microbial.* 54:2492-2499.

Little, B. & Ray, R. 2002. A perspective on Corrosion Inhibition by Biofilms. Corrosion, 58: 424-428.

Lodder, J. (1974). The yeast (2nd ed.) Northern Holland Publishing Co. Amsterdam and American Publishing Co. Incorporated, New York.

Longley, L. E., B. E. Moore & C. A. Sorber (1980). Comparison of Chlorine and Chlorine dioxide. Poll. Control fed., 52: 2098-2105.

Madigan, M. T., Martinko, T. M., Dunlap, P. V. & Clark, D. P. (2009). *Brock Biology of Microorganism* (12th ed.) New York: Pearson Benjamin Cunning, 908-911.

Muthukumar, N., Maruthanmuthu, S., Palaniswany, N. (2007). Role of cationic and nonionic surfactants on biocidal efficiency in diesel-water interface. Colloids Surf B: Biointerfaces, 57: 152-160.

Pope D. H., D. J. Duquette, D. C. Wagner Jr, & A. H. Jamannes 1984. Microbiolly Influenced corrosion, a state of the Art Review. *Material Technology Institute of the Chemical Process Industries.* Columbus, Ohio, p. 76.

Prasad, R. (2000). Assessment and Control of MIC in the oil Industry in the 20th Century, Paper 00390, Presented at NACE International CORROSION 2000 Conference and Exhibition, Orlando, FL. March 26-31.

Puyate, Y. T. & A. Rim-Rukeh (2008). Biocidal efficiency of dissolved ozone, formaldehyde and sodium hypochlorite against total planktonic microorganisms in produced water. J. Applied Sci. 8: 860-865.

Videla, H. A. (1996).Manual of Biocorrosion. CRC Lewis publishers, Florida.

Videla, H. A. 1996. Manual of Biocorrosion. Florida: CRC Lewis Publishers 273p.

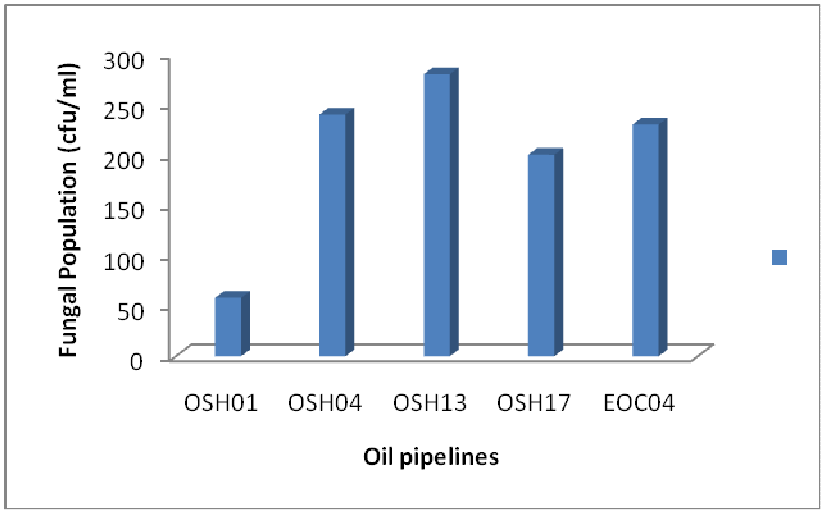

Figure 1: Fungal Populations per Oil Pipeline in Rivers State

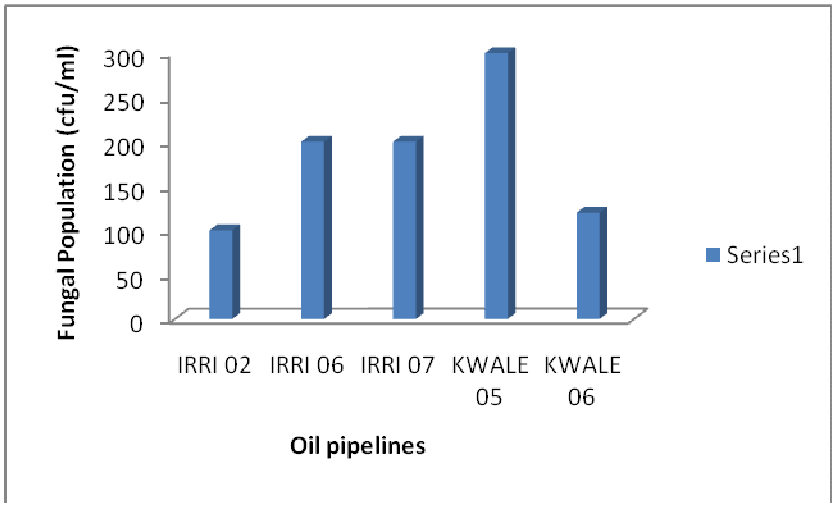

Figure 2: Fungal Populations per Oil Pipeline in Delta State

Table 1: Determination of the MIC of the Tested Biocides against Fungal Isolates by using Colorimetric Measurement at 650nm

	Ozone Concentration (%)						Sodium Hypochlorite Concentration (%)						Formaldehyde Concentration (%)					
Iso	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
1	0.44	0.00	0.00	0.00	0.00	0.00	0.39	0.22	0.10	0.10	0.00	0.00	0.38	0.01	0.00	0.00	0.00	0.00
2	0.66	0.10	0.05	0.02	0.02	0.02	0.39	0.20	0.01	0.01	0.01	0.01	0.28	0.08	0.00	0.00	0.00	0.00
3	0.86	0.06	0.00	0.00	0.00	0.00	0.49	0.12	0.11	0.04	0.02	0.02	0.37	0.00	0.00	0.00	0.00	0.00
4	0.46	0.02	0.01	0.01	0.01	0.01	0.75	0.20	0.07	0.04	0.02	0.02	0.75	0.03	0.00	0.00	0.00	0.00
5	0.51	0.06	0.03	0.02	0.01	0.01	0.39	0.01	0.00	0.00	0.00	0.00	0.38	0.01	0.01	0.01	0.01	0.01
6	0.49	0.19	0.02	0.01	0.00	0.00	0.39	0.20	0.01	0.01	0.01	0.01	0.28	0.08	0.00	0.00	0.00	0.00
7	0.76	0.12	0.03	0.01	0.01	0.01	0.46	0.06	0.04	0.03	0.01	0.01	0.28	0.05	0.01	0.01	0.00	0.00
8	0.60	0.10	0.04	0.04	0.01	0.01	0.48	0.22	0.05	0.02	0.01	0.01	0.90	0.03	0.02	0.02	0.02	0.02
9	0.28	0.13	0.03	0.01	0.00	0.00	0.36	0.04	0.02	0.01	0.01	0.01	0.36	0.03	0.02	0.00	0.00	0.00
10	0.81	0.07	0.02	0.00	0.00	0.00	0.26	0.13	0.04	0.02	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00
11	0.55	0.02	0.00	0.00	0.00	0.00	0.29	0.18	0.00	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.00
12	0.65	0.11	0.07	0.01	0.00	0.00	0.65	0.12	0.02	0.02	0.00	0.00	0.38	0.16	0.02	0.00	0.00	0.00

Iso 1. Verticillum dahlae, Iso 2 Saccharomyces cerevisiae, Iso 3 Penicillium corylophilum, Iso 4 Botrytis cinerea, Iso 5 Fusarium oxysporum, Iso 6 Aspergillus paradoxus, Iso 7 Humicola grisae, Iso 8 Aureobasidium pullulans, Iso 9 Monilia balanitis, Iso 10 Hormoconis resinaea, Iso 11 Aspergillus flavus, Iso 12 Helminthosporium maydis

Table 2 : Efficiencies (%) of the Tested Biocides against Fungal Isolates from Biofilms of oil pipelines

	Ozone Concentration (%)						Sodium Hypochlorite Concentration (%)						Formaldehyde Concentration (%)					
Iso	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
1	0	100	100	100	100	100	0	43.59	74.35	74.35	100	100	0	97.36	100	100	100	100
2	0	84.84	92.42	96.97	96.97	96.97	0	48.71	97.43	97.43	97.43	97.43	0	71.42	100	100	100	100
3	0	93.02	100	100	100	100	0	75.51	77.55	91.83	95.91	95.91	0	100	100	100	100	100
4	0	95.65	97.82	97.82	97.82	97.82	0	73.33	90.67	94.67	97.33	97.33	0	96.00	100	100	100	100
5	0	88.23	94.11	96.87	97.82	97.82	0	94.87	100	100	100	100	0	97.36	97.36	97.36	97.36	97.36
6	0	61.22	95.92	97.95	100	100	0	48.71	97.44	97.44	97.44	97.44	0	71.42	100	100	100	100
7	0	84.21	96.05	98.68	98.68	98.68	0	86.95	91.30	93.47	97.82	97.82	0	82.14	96.42	96.42	100	100
8	0	83.33	96.67	100	100	100	0	54.17	89.58	95.83	97.92	97.92	0	96.67	97.78	97.78	97.78	97.78
9	0	53.57	89.28	96.43	100	100	0	88.89	94.44	97.22	97.22	97.22	0	91.67	94.44	100	100	100
10	0	91.36	97.54	100	100	100	0	50.00	54.62	92.30	100	100	0	100	100	100	100	100
11	0	96.36	100	100	100	100	0	37.92	100	100	100	100	0	100	100	100	100	100
12	0	83.07	89.23	89.23	100	100	0	81.53	96.92	96.92	100	100	0	57.89	94.73	100	100	100

$$E\,\% = \frac{E_{uninhibited} - E_{inhibited}}{E_{uninhibited}} \times 100$$