Response of nitrosomonas, nitrobacter and escherichia coli to drilling fluids

L.O. Odokuma and E. Akponah

ABSTRACT

Inhibition of ammonia oxidation by *Nitrosomonas*, nitrite consumption by *Nitrobacter* and carbon IV oxide evolution by *Escherithia coil* were employed as indices to determine the effect of four drilling fluids (Paradril, IMCO-W, IMCO-O and Gel/KCL/polymer to these bacteria. The percent log survival of these bacteria when exposed to these fluids was also used as an index. Ammonia oxidation was monitored as nitrite accumulation. Both nitrite accumulation and nitrite consumption were determined by coupling of diazotised sulfanilic acid with α-napthylethylene diaminedihydrochloride. Result revealed decreases *in* the percentage log survival with increasing concentration of the drilling fluids and increasing exposure period of the three bacteria. Nitrite accumulation in cultures containing *Nitmsomonas* decreased with increase in concentration and exposure time of the four fluids. Controls (containing Winogradsky Phase I with *Nitrosomonas* and no toxicants) revealed an increase in nitrite accumulation with increase in exposure time. Nitrite consumption in *Nitmbacter* containing cultures decreased with increase in concentration and exposure time to fluids. Controls (containing Winogradsky Phase II with *Nitrobacter* and no toxicants) showed a similar trend but the decrease was not as rapid. Carbon IV oxide evolution in cultures containing *E. coil* generally decreased with increase in concentration and exposure time to fluids. Controls (containing lactose broth with *E. coli* and no toxicant) revealed an increase in CO_2 evolution with increase in exposure time. These results showed that the drilling fluids inhibited the two stages of the nitrification process in the ecosystem and also respiration by *Escherichia coil.* This may affect the agricultural productivity of ecosystems in the Niger Delta with regards to causing dislocation in the nitrogen cycles where nitrifying bacteria participate and the detritus food chain where aerobic) and facultative anaerobes like *E. coil* are important.

INTRODUCTION

In Nigeria, drilling fluids and cuttings are normally discharged into fills and from there they overflow into nearby farms and rivers. These fluids and cuttings discharged untreated into the environment may have adverse effects on the recipient ecosystem. Drilling fluids plumes of turbid water are commonly seen trailing down streams from drilling platforms (Jack *et l,* 1985). Drilling fluids and cuttings may also be introduced into the recipient aquatic ecosystem when sand and silt traps are emptied to make room in fluid pits from the addition of new mud components or when the fluid pits are employed at the end of the drilling operation (Parker and Prebyl, 1984).

Drilling fluids and cuttings constitute a serious threat to the biota of natural ecosystems (Odokuma and Ikpe, 2003). The individual components of the chemical additives in the drilling fluids inhibit the growth of some microbial communities that are important in some of the biogeochemical cycles present in the affected ecosystem, which may affect the agricultural productivity of such ecosystems (Rhodes and Hendricks 1990).

Nnubia and Okpokwasili (1993) have reported that certain Gram-positive bacteria and fungi utilize drilling fluids and cuttings. They also observed a depressed growth of marine bacteria with these fluids to a varying degree. Odokuma and Ikpe (2003) observed that water-based fluids were more biodegradable than oil-based fluids. They ascribed this observation partly to the greater toxicity of oil-based fluids. Studies have shown that the target of toxicant activity on bacterial systems include cell wall, cytoplasmic membrane, enzyme mediated activities and genetic machinery (Stratton and Corke, 1982; Vandermeulen, 1986; Giesy *et aL;* 1988; Xu and Schurr, 1990; Odokurna and Okpokwasili, 2003 a,b). *In* this study the responses of Nitmsomonas, *Nitrobacter* and *Esche,ichia coli* to four drilling fluids, oil-based Paradril synthetic based IMCO-O, and two water-based fluids IMCO-W and Gel/KC1/Polymer were examined. The three bacteria occupy very important regimes in aquatic and terrestrial food chains.

*Corresponding author:
Manuscript received by the Editor June 2, 2005; revised manuscript accepted October. 20, 2006.
Department of Microbiology University of Port Harcourt, Port Harcourt, Nigeria

MATERIALS AND METHODS

Drilling fluids

These were Paradril, IMCO-O, IMCO-W and Gel/KCL/ Polymer. Paradril and Gel/KCL/Polymer were obtained from Magcobar manufacturing Company Nigeria Port Harcourt while IMCO-O and IMCO-W were obtained from the Nigeria Agip Oil Company Port Harcourt Nigeria.

Source of test organisms

Three test organisms; Nitmsomonas, *Nitmbacter* and *Escherichia coli* were employed in this study. They were all isolated from soil within the University of Port Harcourt, Rivers State, Nigeria. Surface soil samples (0-15cm depth) were collected from soil using a sterile auger drill. The soils were transferred into sterile polyethylene sachets and immediately taken to the laboratory for analysis. The methods used for isolation of bacteria (Nitrosomonas and Nitrobacter) from soil were adopted from Coiwell and Zambruski (1972) *Nitrosomonas* was isolated using Winogradsky medium for nitrification phase 1. The medium had the following composition $(NH_4)_2S0_4$, 2.0g; K_2HPO_4, 1.0g;$MgS0_4.7H_20$, 0.5g; NaCl,2.0g; FeSO4.7H20, 0.4g; $CaC0_3$ 0.01, agar 15.0g; distilled water l000ml. *Nitrobacter* was isolated using Winogradsky medium phase 2. It had the following composition, $KN0_2$ 0.lg; Na2-Co3,1.0g; NaC1, 0.5g; FeSO4.7H20, 0.4g agar 15.0g; distilled water l000ml. *Escherichia coli* was isolated using methylene blue agar. The media were sterilized by autoclaving and aseptically dispersed into sterile Petri dishes after cooling to about 45°C. The Petri dishes were then inoculated with and incubated aerobically for 4 days at room temperature (28 ± 2°C) for *Nitrosomonas* and *Nitrobacter* and 18 to 24h at 42°C for *E. coli.* Further identification and characterization of pure cultures of these organisms were undertaken using criteria of Krieg and Holt (1994). The broth media used for isolation of the test organisms also served as diluents for producing the various toxicant concentrations.

Standard inocula

Discrete colonies from each of the different culture media were subcultured into fresh media. These were transferred into slants and stored at 4°C. The slant cultures served as stock cultures. The standard inocula were prepared from the stock cultures. Each of the isolates were picked from the respective stock cultures and incubated at 37°C for 24h. One milliliter was transferred from the respective flasks and ten-fold serial dilutions were made up to 10^{-3}. An amount (0. 1ml) of the 10^{-3} dilutions was plated into appropriate sterile agar plate. Incubation under appropriate conditions of the isolates followed immediately. Plates containing 45-70 colonies were selected for the toxicity test.

Nitrification tests

Nitrification inhibition tests were carried out to determine the toxicant effect on nitrite accumulation by *Nitrosomonas*, nitrite consumption by *Nitrobacter* and evolution of carbon IV oxide by *E. coil*. Five logarithmic concentrations of each of the drilling fluids: 0.01, 01, 1.0, 10.0 and l00.0mg/L were prepared using appropriate broth: Wiriogradsky phase 1, Winogradsky phase II and lactose broth were used as diluents in tests with *Nitrosomonas, Nitmbacter* and *E. coil* respectively. One hundred milligrams of each of the drilling fluids were weighed. The volume of this weight was noted. This volume was then transferred into a liter conical flask. For water-soluble drilling fluids the volume was then made up to l000ml with diluents (broth). Subsequent concentrations, 10,1.0,0.1 and 0.01 mg/l were obtained by further ten-fold serial dilution. The procedure for water insoluble fluids differed slightly. The volume equivalent of 100mg of mud was transferred to a 1L flask and the volume made up to l000ml with diluent. The same was repeated to achieve 10, 1.0, and 0.0lmg/l by dividing the equivalent volume of l00mg/l of mud by 10, 100, 1000 and 10,000 respectively and making up the volume to l000iril with diluent. No solvent was employed to dissolve the water insoluble drilling muds. This was done deliberately to avoid synergistic or antagonistic effect of solvent.

The nitrite accumulation test was adapted from APHA (1998). The standard inoculum (0.lml) of *Nitrosomonas* was added to each of the toxicant concentrations contained in 250m1 Erlenrneyer flask. Nitrite concentration accumulated by *Nitrosomonas* was determined by coupling of diazotised sulfanilic acid with a-napthyl-ethylene-diamine dihydrochioride (NED) (APHA, 1998). Controls containing Winogradsky phase 1 broth and organism without toxicant were treated in the same manner. Increase in the concentration of nitrite with time indicated a positive result (the ability of the organism to convert ammonia to nitrite).

The nitrite consumption test was adapted from (APHA, 1998). The standard inoculum (0. 1ml) of *Nitrobacter* was added to triplicate sets of the toxicants concentration as in nitrite accumulation tests. The mixture was incubated at room temperature (28 ± 2°C) for 72h. At exposure time of 0h, 2h, 3h, 4h, 8b, 12h, 24h, 36b and 48h nitrite content was determined as in nitrite accumulation test.

Carbon 1V oxide evolution test

The method of Allen *et al.* (1985) was adapted for the determination of evolution of carbon *N* oxide by *E. coli*. The standard inoculums (0.Ml) or *E.* coli was inoculated into each 250m1 Erlenmeyer flasks containing sterile lactose broth with various toxicant concentrations. The flasks were corked with sterile wooden corks with holes through which glass delivery tubes were passed into another 250m1 Erlenmeyer flask containing 200m1 of O.O1M calcium hydroxide

solutions. Incubation followed immediately at 37°C for 48h. At exposure time of 0, 1, 2, 3, 4, 8, 12, 24 and 48h, 10ml of Ca $(OH)_2$ were collected from each of the receiving flasks and 0.02m tetraoxosulphate IV acid (H_2S0_4) was used to titrate against the base. Methyl-red was used as indicator. The amount of carbon IV oxide (in moles) evolved was equated to the number of moles of base used in titration. It was converted to volumes by multiplying by 22.4dm^3. The stoichiometric equation of the reaction is given below;

$Ca(OH)_2(aq) \div 2CO_2 \rightarrow Ca(HCO_3)2(aq)$

$Ca(HCO_3)_2(aq) \div H_2SO_4(aq) \rightarrow CaSO_4(s) + 2H_2O + CO_2(g)$

From step II, 1 mole of CO_2 = 1 mole of $CaHCO_3(g)$

Mole of $Ca(HCO_3)_2 = C_AV_A/V_B = M_C$

where

C_A = Concentration of acid 0.02

V_A = Volume of acid used in titration

V_B = Volume of base used = 10ml

M_C moles of CO_2

Actual volume of CO_2 = Mc x 22.4dm^3

Percent log survival test

Mortality was another parameter employed in assessing the toxicity of the drilling fluids to the three organisms. The method of Okpokwasili and Odokuma 1996a,b) adapted from Williamson and Johnson (1981) was employed as an additional index for assessing bacterial response to these drilling fluids. The various drilling fluid concentrations were inoculated with 5ml of broth culture (Standard inoculums) of the bacteria. The drilling fluid concentrations were in solid media selective for the particular bacteria. The broth cultures were inoculated using the spread plate method (APHA, 1998). The solid cultures (plates) were exposed for periods similar to those employed Ofor monitoring enzyme mediated activities. Plates were incubated at room temperature and those containing between 30 and 300 colonies were selected and counted 48h later. The percent log survival was calculated by obtaining the log of the count in each toxIcant concentration, dividing by the log of the count in the zero toxicant concentration and multiplying by 100 (Williams and Johnson, 1981).

RESULTS

There was a decrease in the percent log survival of *Nitrosomonas* with increasing contact time (exposure period) and concentration of drilling fluid (Fig la — 1d). Mortality was greatest at the 36 and 48h exposure period for all system containing fluids. The effect was greatest with Paradril IMCO-O, Gel/KCL/ Polymer and least with IMCO-W. Controls in which no fluid was present showed a constant population of viable cells (absence of mortality)through out the 48h exposure period.

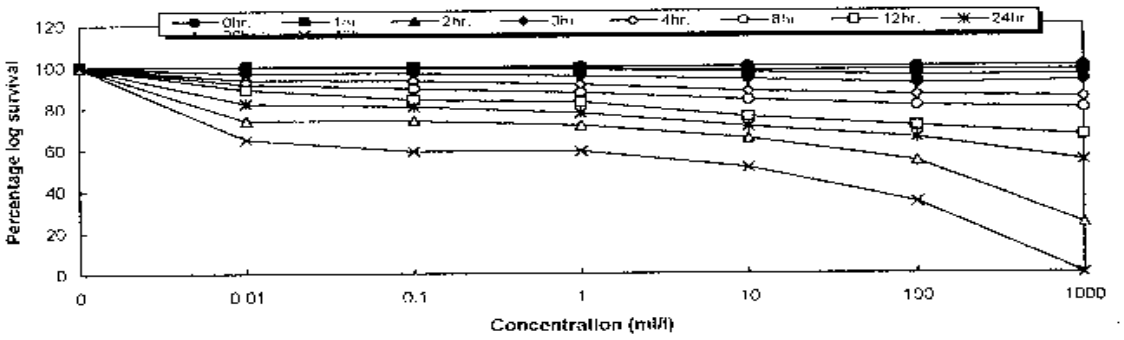

Fig. 1a: Effect of IMCO-W on percent log survival of *Nitrosomonas*

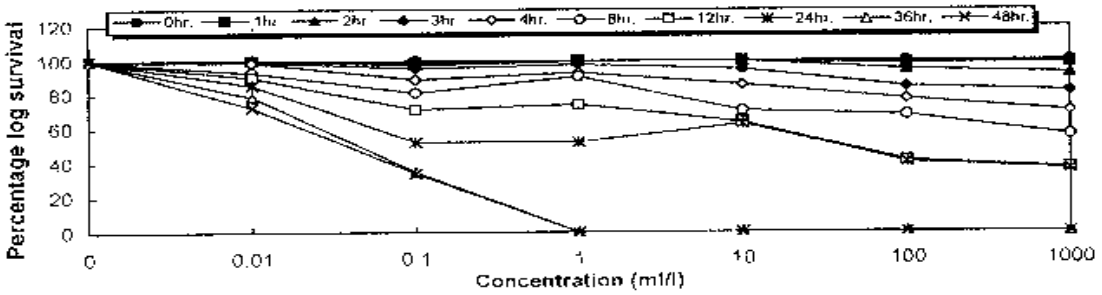

Fig. 1b: Effect of Paradril on percent log survival of *Nitrosomonas*

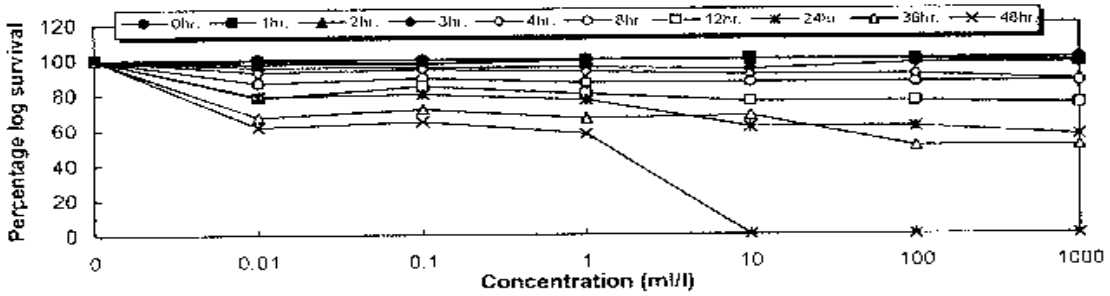

Fig. 1c: Effect of Gel/KCl/Polymer on percent log survival of *Nitrosomonas*

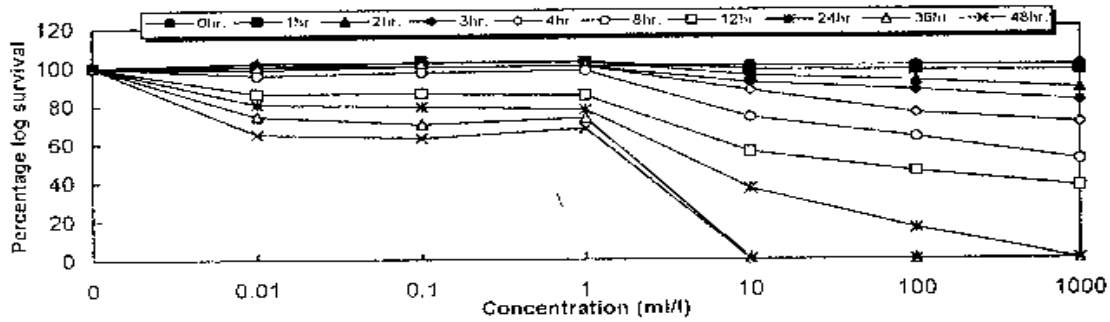

Fig. 1d: Effect of IMCO-O on percent log survival of *Nitrosomonas*

Nitrobacter displayed a similar trend as that of *Nitrosomonas* when exposed to the four drilling fluids (Fig 2a-2d). However mortality was evident at lower contact times of 12 and 24h in IMCO-O, IMCO-W and Gel/KCL/Polymer. These results suggested that *Nitrobacter* might have been more sensitive to the fluids than Nitmsomonas. Controls containing no drilling fluid showed a constant viable cell population, which was independent of contact time.

The effect of the fluids on the percent log survival of *Escherichia coil* (Fig 3a-3d) revealed a similar trend to that of *Nitrosmonas* and *Nitrobacter.* A decrease in the percent log survival of the organism with increase in exposure time and concentration of the fluids was observed. As in *Nitrosomonas* the effect was more evident during the 36 and 48h contact times. The contact time did not affect the viable cell count of *E. coil* in controls.

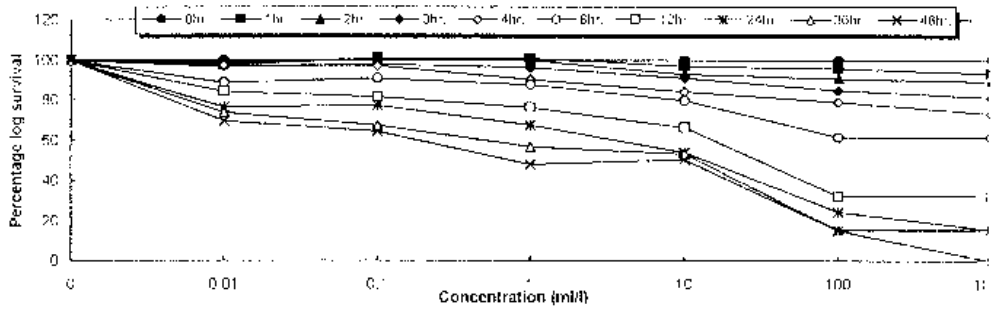

Fig. 2a: Effect of Paradril on percent log survival of *Nitrobacter*

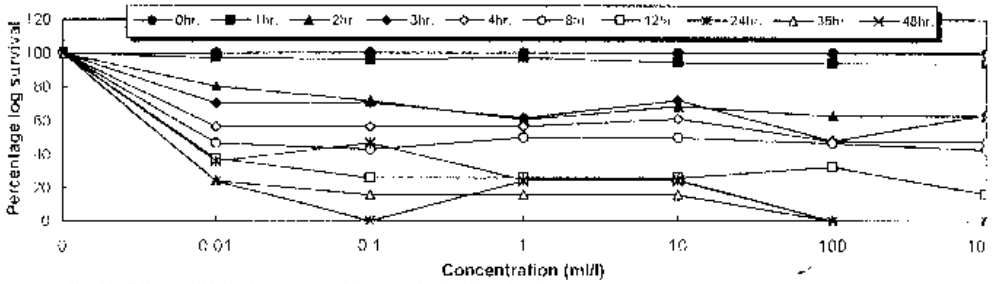

Fig. 2b: Effect of IMCO-O on percent log survival of Nitrobacter

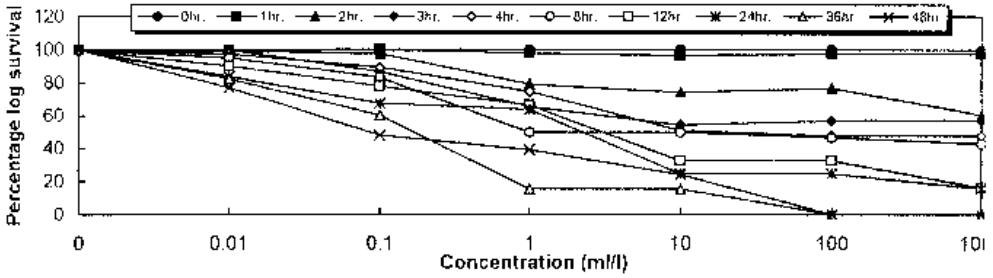

Fig. 2c: Effect of IMCO-W on percent log survival of Nitrobacter

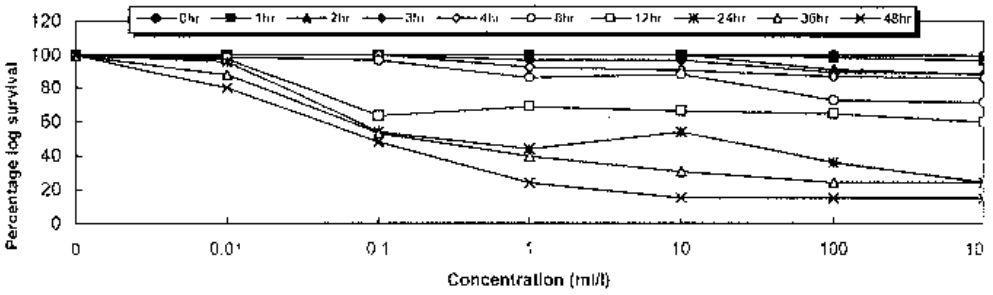

Fig. 2d: Effect of Gel/KCl/Polymer on percent log survival of Nitrobacter

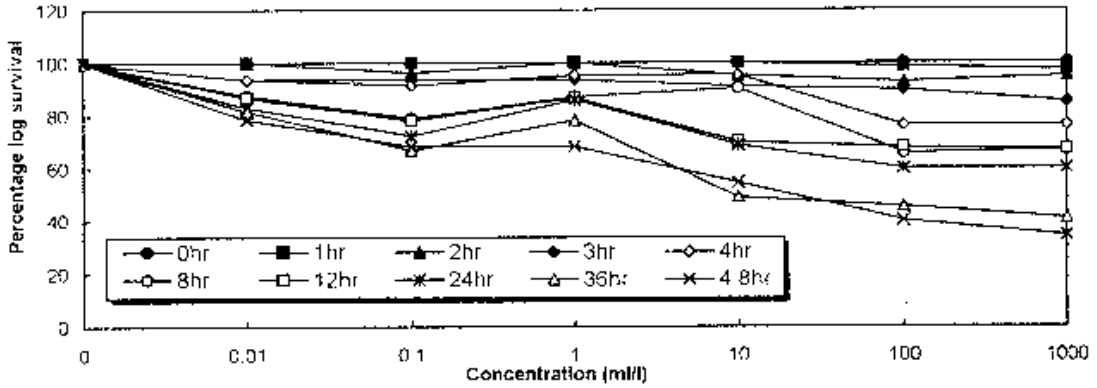

Fig. 3a: Effect of Gel/KCl/Polymer on percent log survival of Escherichia coli

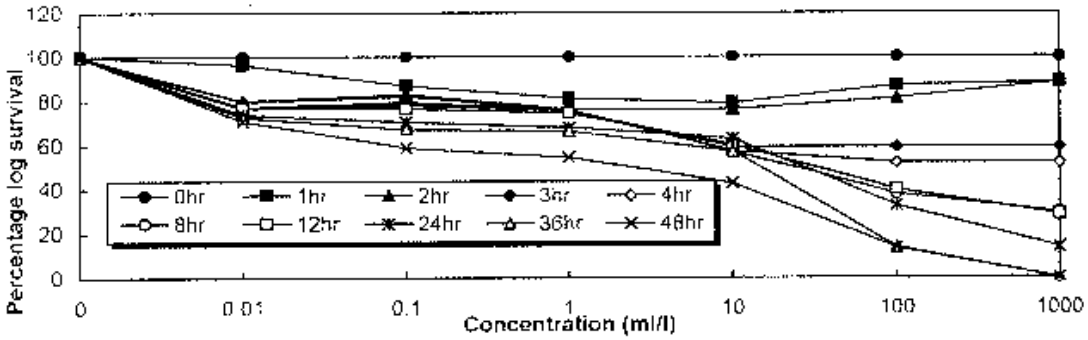

Fig. 3b: Effect of Paradril on percent log survival of *Escherichia coli*

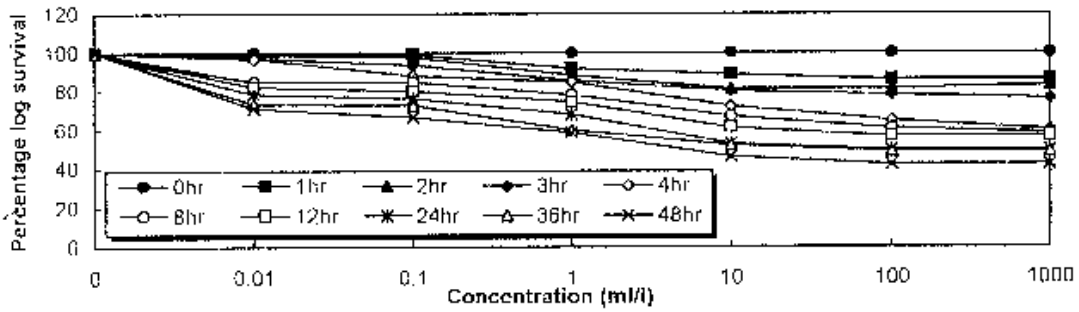

Fig. 3c: Effect of IMCO-O on percent log survival of *Escherichia coli*

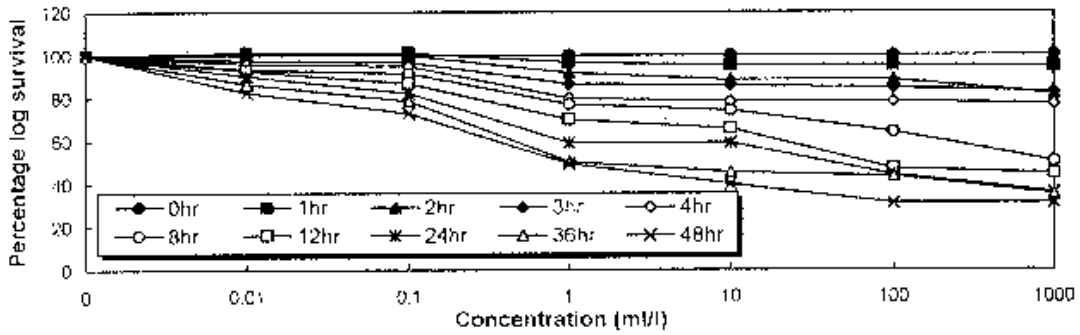

Fig. 3d: Effect of IMCO-W on percent log survival of *Escherichia coli*

In Fig 4a-4d nitrite accumulation (oxidation of ammonia to nitrite) by *Nitrosomonas* is presented. In controls nitrite accumulation increased from Oh to 24h and decreased to levels similar to the 2,3, and 4h during the 36 cell 48h. Nitrite accumulation by *Nitrosomonas* decreased from 2h to the 48h exposure period for all toxicant concentration. Results showed that nitrite accumulation by *Nitrosomonas* was inhibited by Paradril. A similar effect of IMCO-O (Fig 4b) on *Nitrosomonas* was observed. With IMCO-W the effect on nitrite accumulation by *Nitrosomonas* was not slightly modified. Like the previous two toxicants between 1h and 2h there was increase in nitrite accumulation especially at concentration 0.01 and 0.1 and 1.0. However unlike the Paradril and IMCO-O which experienced a

decrease in nitrite accumulation in all concentrations after 2H IN IMCO-W there was a slight decrease till 4h and an increase in the 8h at lower concentration of 0.01, 0.1 and 1.0mg/i which was followed by a decrease till the 48h. The effect of Gel/KCL/Polymer on nitrite accumulation by *Nitrosomonas* followed a similar trend with that of Paradril and IMCO-O. There was a decrease in nitrite accumulation with time for all exposure periods till 48h especially after the 2h indicated that nitrite accumulation by *Nitrosomonas* was inhibited with time by the four toxicants.

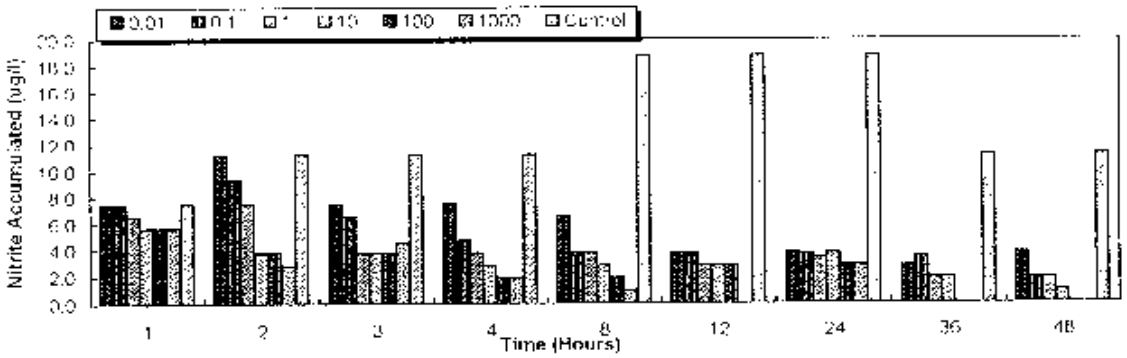

Fig. 4a: Effect of Paradril on *Nitrosomonas* as a function of nitrite accumulation

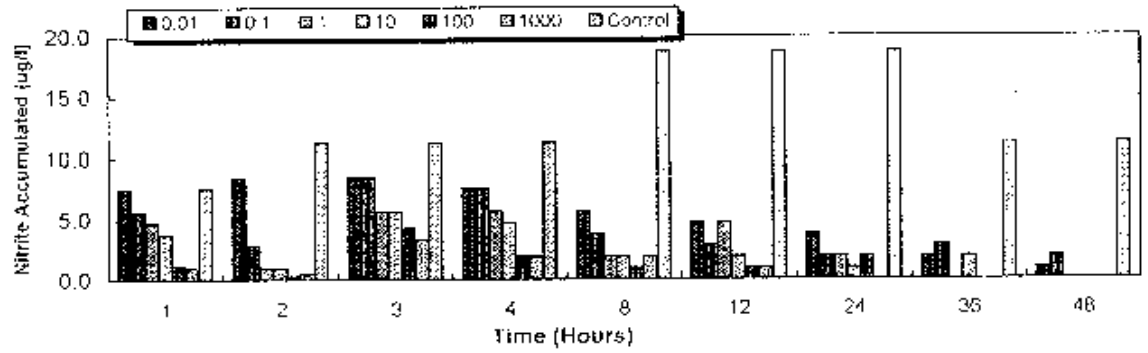

Fig. 4b: Effect of IMCO-O on *Nitrosomonas* as a function of nitrite accumulation

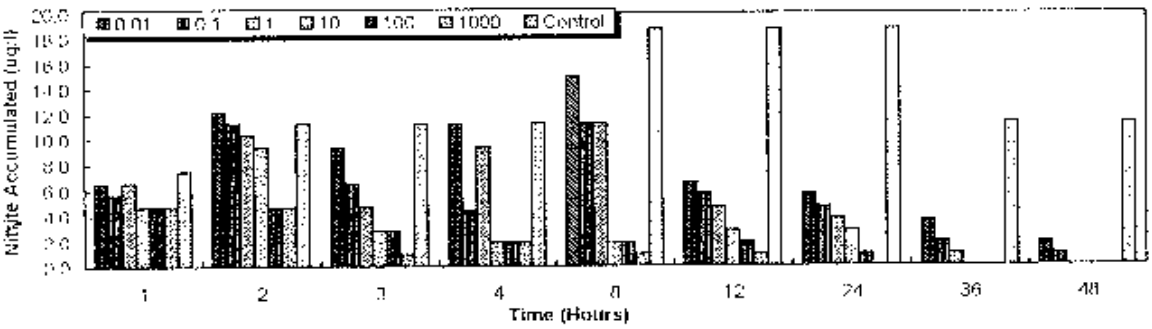

Fig. 4c: Effect of IMCO-W on *Nitrosomonas* as a function of nitrite accumulation

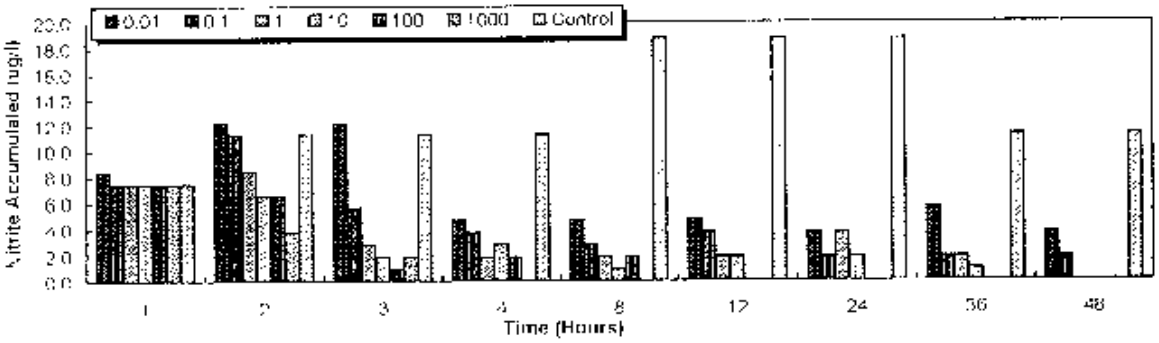

Fig. 4d: Effect of Gel/KCl/Polymer on *Nitrosomonas* as a function of nitrite accumulation

In Fig 5a-5d nitrite consumed (oxidation of nitrite to nitrate) by *Nitrobacter* is presented. In the controls there was a decrease in nitrite consumed with time. This became apparent from 2h to the 48h exposure period. However values ranged from 28µg/l at 2h to 3µg/l by the 48h. Between 3h and 12h exposure period nitrite consumed during control ranged between 14µg/l to l8µg/l. Nitrite consumed decreased with time at all concentrations when *Nitrobader* was exposed to IMCO-W (Fig 5a). Nitrite consumed was greater in cultures of control than in IMCO-W containing cultures. Similar observations were obtained when *Nitrobacter* was exposed to IMCO-O, Paradril and Gel/KCL/Polymer.

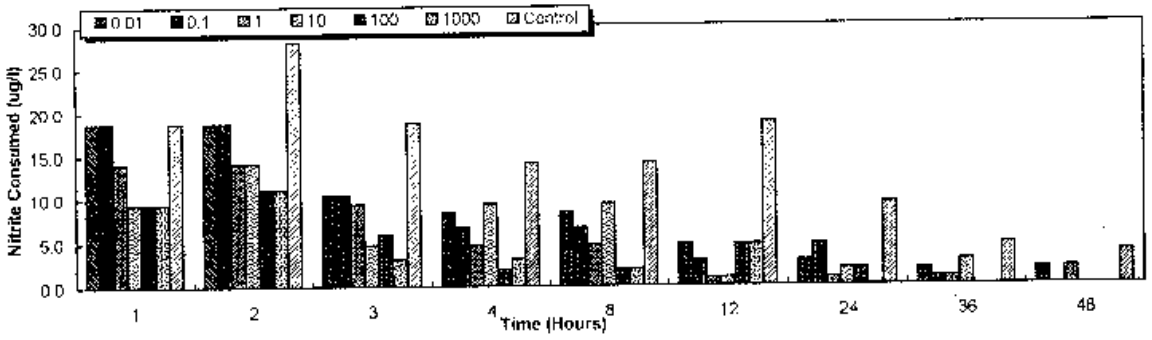

Fig. 5a: Effect of IMCO-W on *Nitrobacter* as a function of nitrite consumption

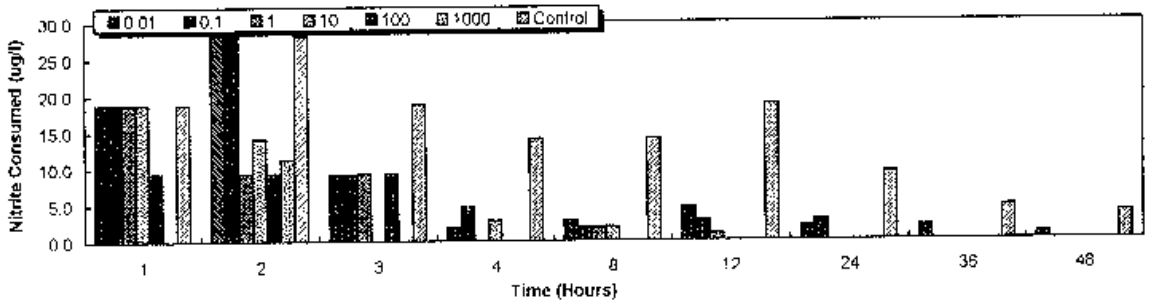

Fig. 5b: Effect of IMCO-O on *Nitrobacter* as a function of nitrite consumption

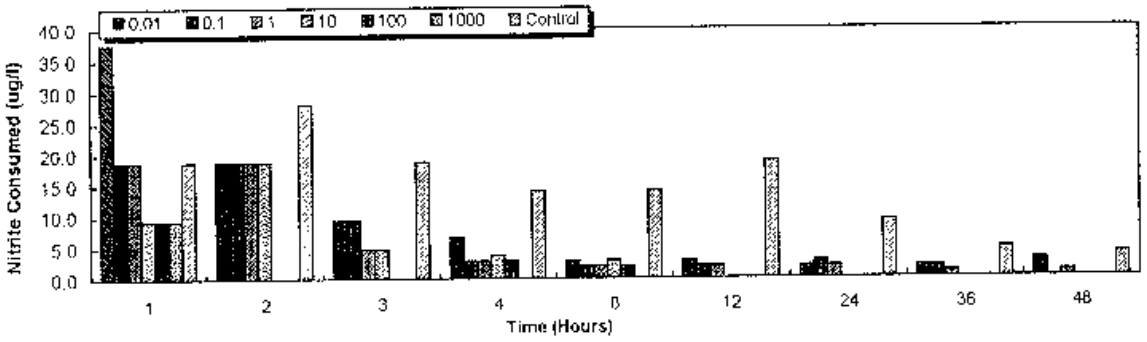

Fig. 5c: Effect of Paradril on *Nitrobacter* as a function of nitrite consumption

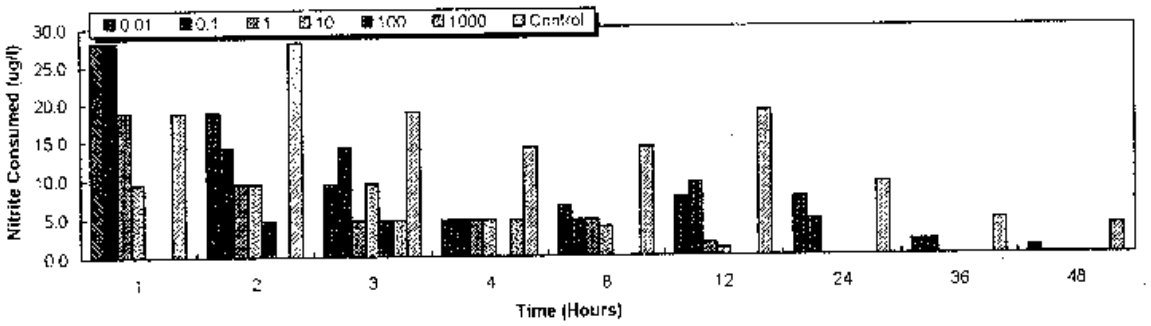

Fig. 6d: Effect of Gel/KCl/Polymer on *Nitrobacter* as a function of nitrite consumption

In Fig 6a-6d the effect on the toxicants on carbon IV dioxide evolution by *Escherichia coli* are presented. Results showed that in the control there was an increase in the amount of CO_2 evolved with exposure time. This was evident in the 8h to the 36h. By the 48h CO_2 evolution decreased. Between 1h and 4h, CO_2 evolution in the control cultures were constant at 0.02 dm^3.

This result showed that there was a lag period of about 6h before *E. coli* became fully adapted to the environment and started respiring normally resulting in an increase in the CO_2 produced. In Fig 6a, CO2 evolution decreased with time especially at the high concentrations (10,100 and 1000 mg/l) of Paradril. At lower concentrations (0.01 and 0.1 mg/l) of Paradril during the 24 and 36b CO_2 evolution increased slightly. These results indicated that Paradril generally inhibited respiration by *E. coli* especially at high concentrations of 10,100 and 1000 mg/l, respectively.

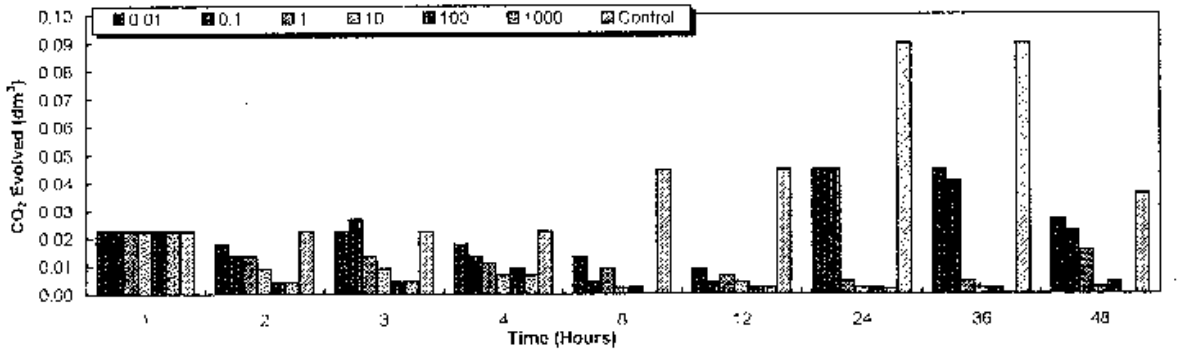

Fig. 6a: Effect of Paradril on *Escherichia coli* as a function of CO_2 evolution.

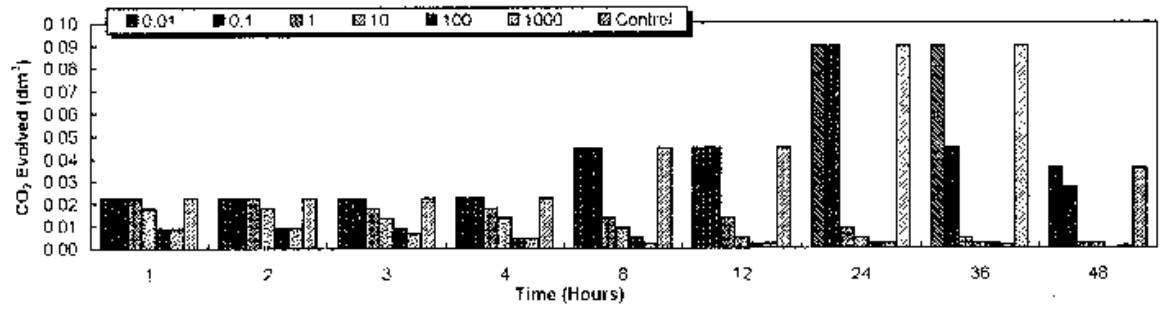

Fig. 6b: Effect of Gel/KCl/Polymer on *Escherichia coli* as a function of CO_2 evolution.

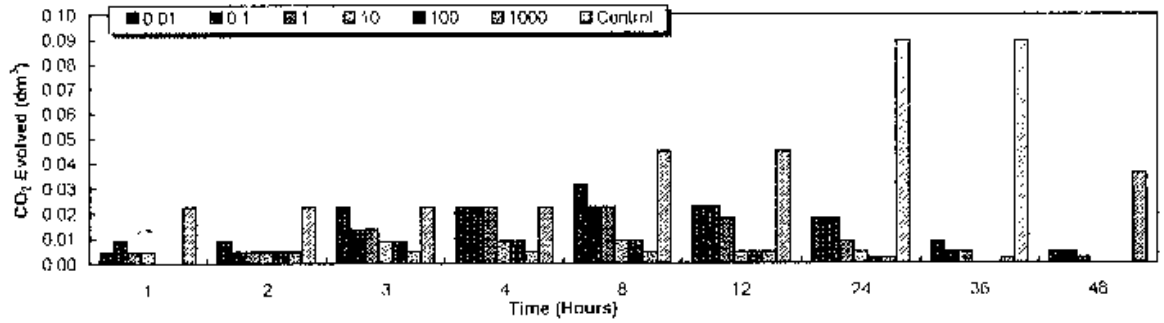

Fig. 6c: Effect of IMCO-O on *Escherichia coli* as a function of CO_2 evolution.

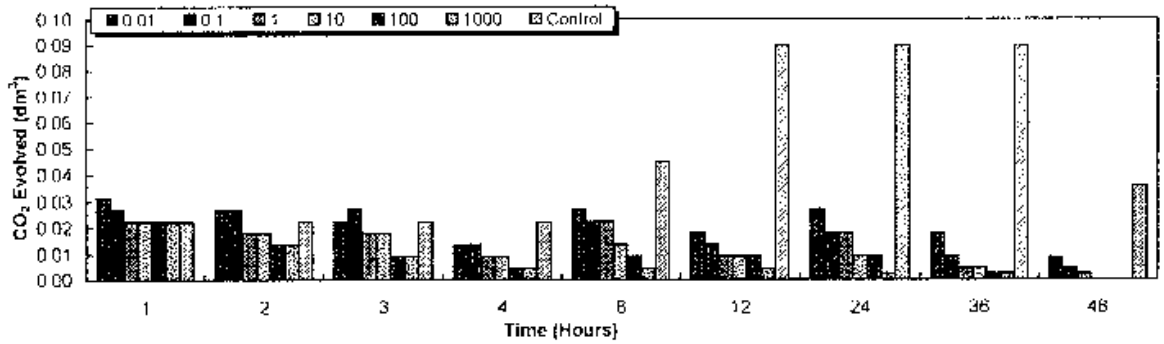

Fig. 6d: Effect of IMCO-W on *Escherichia coli* as a function of CO_2 evolution.

Lower concentrations of Paradril 0.01 and 0.1mg/l seemed to stimulate respiration after 24 to 36h exposure suggesting probable adaptation to the toxicant at these concentrations.

Similarly result was obtained when *E. coli* was exposed to Gel/KCL/Polymer. At high concentration (10,100 and 1000 mg/l) of this toxicant CO_2 evolution decreased with time throughout the 48h exposure period. However at concentration of 0.01 and 0.1mg/l CO_2 evolution increased at the 8h (0.045 dm^3) and reached its peak at the 24 and 36h (0.09 dm^3) then declined at the 48h (0.035 dm^3). These results were identical to that of the control cultures indicating that these concentrations of toxicant were stimulation to CO_2 evolution by *E. coil.*

The effect of IMCO-O (Fig 6c) on CO_2 evolution by *E. coil* was not exactly similar to that of the other two toxicants. There was a slight increase in CO_2 evolution with time (lh to 3h) from less than 0.01dm^3 to 0.02dm^3. This value was maintained till the 8h then it reduced from this time to the 48h. When comparing these values with those of the control CO_2 evolution was very much inhibited by this toxicant. There was a decrease in CO_2 evolution with time when E. coil was exposed to IMCO-W. However the rate of decrease was not as much as that of Paradril.

DISCUSSION

The study revealed bactericidal properties of the drilling fluids evident from decrease in the percentage survival of the three bacteria with increasing contact time (exposure period) and concentration of the drilling fluid. Similar observations have been made by Okpokwasili and Odokuma (1997) who observed a decrease in percent log survival with increase in contact time and concentration when *Nitrobacter* was exposed to three oil spill dispersants and five domestic detergents. Odokuma and Ogbu (2002) observed that toxicity factors of dispersants were greater than one when these dispersants were exposed to *Bacillus* and *Escherichia coil* indicating that the bacteria were tolerant to the oil spill dispersants. Okpokwasili and Odokurna (1997) suggested that the toxicity of the dispersants to *Nitmbacter* may have resulted from dispersant effect on any of the target sites of the organisms. The site of action of a toxicant being a function of the nature, concentration and contact time of the toxicant (Odokuma and Okpokwasili, 2003 a,b). The present study revealed that the drilling fluids caused cell mortality leading to a reduction in the viable cell count. This effect though exhibited by all three bacteria appeared to be greater with *Nitrobacter.*

A decrease in some metabolic activities of the bacteria (nitrite accumulation by *Nit rosomonas,* nitrite consumption by *Nitrobacter* and respiration by *E. coil)* were some of the toxic effects displayed by the drilling fluids. The decrease in nitrite consumption by *Nitrobacter,* in response to toxicants has been attributed to the sensitivity of the constitutive enzyme, the nitrite enzyme mediating the oxidation of nitrite to nitrate in *Nitrobacter* (Odokuma and Okpokwasili 2003 a,b).

Inhibition of the nitritase enzyme by toxicants apart from cell wall disruption may be due to the high permeability of *Nitrobacter's* outer membrane (Madigan *et el,* 1997). The membrane being the site of the nitritase enzyme complex mediated respiration (Madigan *et* al., 1997) thus their inhibition affected the respiration process. Though *Nitrobacter* and *Nit rosomonas* are both Gram negative, the location of the *ammonia* monooxygenase enzyme responsible for the oxidation of ammonia to nitrite (determined by nitrite accumulation) in *Nitrosomonas* is not resident in the cell membrane (Nester *et* a!., 1998). Thus the interaction with the drilling fluids and the monooxygenase enzyme is reduced compared with that of the nitritase enzyme in *Nitrobacter.* This may have contributed to the slightly lesser effects of the fluids on the percentage log survival of *Nitrosomonas.* However, the decrease in nitrite accumulation with increasing concentration and contact *time* with fluids suggested an inhibition of the ammonia monooxygenase enzyme. Inhibition of carbon IV oxide evolution in *E. coil* by the fluids was due to prevention of the fermentation of lactose present in the lactose broth (Dutton *et al.,* 1990).

CONCLUSION

Drilling fluids inhibited two stages of nitrification (ammonia oxidation and nitrite oxidation) by *Nitrosomonas* and *Nitrobacter* respectively. The fluids also inhibited respiration in *Escherichia coil.* The fluids decreased the growth of all the test organisms. This may result in the reduction of primary productivity of affected ecosystems due to dislocation in the nitrogen cycle and food chains where aerobic and facultative anaerobic bacteria such as nitrifying organisms and *E. coil* feature prominently.

REFERENCES

Allen, SA.H., Parkinson, M.G.S. and Quarmby, C. (1984). *Chemical analysis of ecological materials,* Blackwell Scientific Publication, Oxford, London : 551-553.

American Public Health Association (APHA) (1998). *Standard methods for the examination of water and waste-water,* 20th ed. Washington DC.

Coiwell, R,R and Zambruski M.S. (1972). *Methods of aquatic microbiology,* University Park Press, Baltimore.

Dutton, R.J, Bitton, G., Koopman, E and Agami,O. (1990). Effects of environmental toxicants on enzymes biosynthesis. A: comparison of 3-galactosidase and a-glucosidase and tryptophanase. *Archives of Environmental Contamination and Toxicology* 4: 101-109.

Giesy, J.P. Graney, R.L. Newsted, J.P. Rosui, C.J. Benda, A., Kreis, R.G. and Horvath, F.J. (1988). Comparison of three sediment bioassay methods using Detroit River Sediments. *Environment Toxicology Chemical* '7:485-498.

Jack, H. ,Thompson J.R., Eugene, A.S. and Thomas, J.B. (1985). Effects of drilling muds on screen species of reef-building corals as measured in the field and laboratory In: *Pmceedings of the 1985 International Seminar of Nigerian National Petroleum Corporation* Lagos.

Madigan, M.T., Martinko, J.M. and Parker J. (1997). *Brock biology of Microorganisms*, Prentice - Hall International UK Limited, London.

Nester E.W., Roberts, C.E., Pearsall N.N., Anderson, D.G and Nester M.T (1998).*MIcro biology :a human perspective* WCB McGraw-Hill, Boston, Massachusetts.

Kreig, N.R. and Holt, J.G. (1994). *Bergeys manual of systematic bactenology*. Williams and Wilkins Baltimore Ltd.

Kpokwasili, G.C. and Odokurna L.O. (1996a) Response of *Nitrobacter* to toxicity of drilling chemicals. *Journal of Petroleum Science and Engineering* 16:81-87.

Okpokwasili, G.C. and Odokurna L. O(1996b). Tolerance of *Nitrobacter* to toxicity of hydrocarbon fuels. *Journal of Petroleum Science and Engineering* 16:89-93.

Okpokwasili, G.C. and Odokuma, L.O. (1997). Response of *Nitrobacter* to toxicity of oil field dispersants and domestic detergents. *Tropical Freshwater Biology* 6:65-74.

Odokunia, L.O. and Ogbu, H.J. (2002). Tolerance of bacteria and crustaceans to oil spill dispersants. *African Journal of Applied Zoology and Environmental Biology* 4:50-55

Odokuina, L.O. and Ikpe, M.D. (2003). Role of composition on the degradability and toxicity of drilling muds. *African Journal of Applied Zoology and Environmental Biology* 5:6-13.

Odokuma, L.O. and Okpokwasili, G.C. (2003a). Bacterial en~rme biosynthesis inhibition; a tool for ecotoxicity assay. *Global Journal of Pure and Applied Science 9(3):* 311-318.

Odokuina, L.O. and Okpokwasili, G.C. (2003b). Response of microbial enzyme synthesis to toxicity of weathered and biodegraded oils. *Global Journal of Pure and Applied Science* 9(4): 465-474.

Parker, C.E. and Prebyl, E.J. (1984). Assessment of bacterial ATP response as a measurement of aquatic toxicity In: *Toxicity Screening Procedures Using Bacterial Systems. (*Lins D. and Dutka, B.J. eds), Dekker, New York.

Rhodes, A.N. and Hendricks, C.W. (1990). A continuous flow method for measuring effects of chemicals on soil nitrification. *Toxicology Assessment* 5: 77-89.

Stratton, G.W. and Corke, C.T. (1982). Toxicity vs. mutagenicity of some crude oils, distillates and their water soluble fractions. *Water Research* 19: 1285 — 1289.

Vanderineulen, J.H. (1986). Altered grazing patterns of an experimental copepod-alga ecosystem exposed to naphthalene and Kuwait crude oil. *Bulletin of Environmental Contamination and Toxicology* 36: 260-266.

Williamson, *K.J.* and Johnson, O.G. (1981). A bacterial bioassay for assessment of wastewater toxicity. *Water Research 15:* 383-190.

Xu, H.H. and Schurr, KM. (1990). Genotoxicity studies on sediments using a modified SOS chromotest. *Toxicology Assessment* 2:79 – 87.