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Full Length Research Paper

Response of *Nitrosomonas*, *Nitrobacter* and *Escherichia coli* to drilling fluids

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Inhibition of ammonia oxidation by *Nitrosomonas,* nitrite oxidation by *Nitrobacter* and carbon IV oxide evolution by *Escherichia coli* were employed as indices to determine the effect of four drilling fluids (Paradril, IMC0-W, IMC0-O and Gel/KCL/polymer to these bacteria. The percentage survival (determined by aerobic spread plate count) of these bacteria when exposed to these fluids was also used as toxicity index. Ammonia oxidation (nitrite accumulation) and nitrite oxidation (consumption) were determined by coupling of diazotised sulfanilic acid with α-napthyl-ethylene diamine dihydrochloride. Carbon IV oxide evolution was monitored by passing air from inoculated lactose broth into calcium hydroxide solution and titration with sulphuric acid. Toxicity indices were determined throughout the following exposure period (0, 1, 2, 3, 4, 8, 12, 24, 36 and 48 h) in static shake flask systems. Percentage survival decreased with increasing concentration of the drilling fluids and increasing exposure period of the three bacteria especially at high concentrations (10.0, 100 and 1000 mg/l). At lower concentrations (0.01, 0.1 and 1.0 mg/l) some fluids stimulated bacterial growth. Controls revealed bacterial growth through out the exposure period. Nitrite accumulation by *Nitrosomonas*, nitrite consumption by *Nitrobacter* and carbon IV oxide evolution by *E. coli* decreased with increase in concentration and exposure time of the four fluids. Controls revealed an increase in these indices with increase in exposure period. Results showed that the drilling fluids inhibited the two stages of the nitrification in the nitrogen cycle and respiration (by *E. coli*). This may affect the agricultural productivity of ecosystems in the Niger Delta where they are employed by causing dislocations in the nitrogen cycle where nitrifying and aerobic bacteria participate.

Key words: Nitrite accumulation, nitrite consumption, respiration, drilling fluids.

INTRODUCTION

In the Niger Delta, drilling fluids and cuttings are sometimes discharged into fills and from where they overflow into nearby farms and rivers. Small amounts are re-injected into special cutting re-injection (CRI) wells while lesser amounts are treated in thermal desorption units (TDU). 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 al., 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). Odokuma and Okpokwasili (1996, 2003a) have shown that drilling fluids inhibited the oxidation of nitrites to nitrates the second step in the nitrification process of the nitrogen cycle. 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

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fluids. They ascribed this observation partly to the greater toxicity of oil-based fluids. Okpokwasili and Odokuma (1996) observed that some drilling chemicals such as Huile clean (Clean oil), Chaux lime (Calcium Hydroxide), Carbotrol A9 (organic polymer blend containing methanol and asphalt) generally inhibited nitrite utilization and decreased the growth rate of *Nitrobacter.* They also observed that certain concentrations (10 mg/L) of Carbotrol A9, Chaux lime and Huile clean stimulated the growth of this organism as exposure time increased from 0 - 4 h. Odokuma and Okpokwasili (2003a) have shown that these drilling chemicals inhibited the synthesis of nitritase enzyme in *Nitrobacter* during a 4 h exposure period, however, only Chaux lime inhibited synthesis of the intracellular enzyme beta-galactosidase in *Escherichia coli.* These chemicals did not inhibit both the biosynthesis of extracellular enzymes tryptophanase by *E.coli* and alpha-glucosidase by *Bacillus thuringensis*. These results indicated that the response of an organism to a toxicant is also dependent on the genetic constitution of the organism and the gene operon mediating enzyme biosynthesis. Studies have confirmed 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; Odokuma and Okpokwasili, 2003a,b). Odokuma and Okpokwasili (1996) have suggested that the toxicity of drilling chemicals were also dependent on their water solubility and chemical composition. For instance Chaux lime was a solid and was water-soluble. It mainly consisted of calcium hydroxide which when dissolved in water raised the pH to 14. High pH levels have been known to inhibit fungal growth and affect protein integrity. Carbotrol A9 and Huile clean were liquids and water-soluble. Carbotrol A9 was an organic polymer containing methanol and asphalt in mineral oil. Its high toxicity was linked to the toxicity of its constituent chemical entities.

In this study the response of *Nitrosomonas, Nitrobacter* and *E. coli* to four drilling fluids, oil-based Paradril a synthetic based IMC0-O, and two water-based fluids IMCO-W and Gel/KCl/Polymer commonly employed in the Niger Delta were examined. The three bacteria occupy very important niches in aquatic and terrestrial food chains. Both *Nitrosomonas* and *Nitrobacter* are aerobic organisms and are responsible for ammonia and nitrite oxidation (nitrification) respectively in the nitrogen cycle. *E. coli* is a facultative anaerobe and plays an important role in the denitrification (reduction of nitrates to nitrites) process in the nitrogen cycle. However, in an aerobic environment *E. coli* respire aerobically producing carbon dioxide as by-product. The Niger Delta is an area where large-scale up-stream and down-stream petroleum activities occur consists of very sensitive habitats such as freshwater swamps, brackish water mangrove swamps and rainforests. Thus the improper application and disposal of drilling fluids may pose a significant stress on the micro biota of these habitats thereby affecting important biogeochemical cycles contributing to maintaining the integrity of such ecosystems. It is thus important to examine the response (effect on nitrification, aerobic respiretion and mortality) of these organisms to these drilling fluids so that less toxic and more readily biodegradable fluids may be developed especially if current ones in use are toxic, persistent and thus do not meet regulatory requirements.

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; *Nitrosomonas, Nitrobacter* and *E. coli* were employed in this study. They were all isolated from soil within the University of Port Harcourt, Rivers State, Nigeria. Surface soil samples (0-15 cm 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 Colwell and Zambruski (1972). *Nitrosomonas* was isolated using Winogradsky medium for nitrification phase 1 ($.NH_4)_2SO_4$, 2.0 g;K_2HPO_4, 1.0 g;$MgSO_4.7H_2O$, O.5 g; NaCl,2.0 g; $FeSO_4.7H_2O$, 0.4 g; $CaCO_3$ 0.01, agar 15.0 g; distilled water 1000 ml). *Nitrobacter* was isolated using Winogradsky medium phase 2 (KNO_2 0.1 g; Na_2-Co_3,1.0 g; NaCl, 0.5 g; $FeSO_4.7H_2O$, 0.4 g agar 15.0 g; distilled water 1000 ml). *E. 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 24 h 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 sub-cultured 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 24 h. One millilitre was transferred from the respective flasks and ten-fold serial dilutions were made up to 10^{-3}. An amount (0.1 ml) 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 toxi-

Table 1a. Effect of IMCO-W on survival of Nitrosomonas.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.0	50.0	50.0	48.2	53.6	45.5	40.9	34.5	27.2	19.1
0.1	50.0	50.0	48.2	48.2	46.4	41.8	35.4	32.7	27.2	14.5
1	50.0	48.2	47.2	45.5	42.7	38.2	33.6	28.2	24.5	14.5
10	50.0	46.4	44.5	48.5	37.2	32.7	24.5	20.9	18.2	10.0
100	50.0	46.4	41.8	44.3	34.5	29.1	20.0	16.4	10.9	8.5
1000	50.0	46.4	42.7	46.4	32.7	27.2	16.4	10.0	9.2	5.2
Control 1	50.0	50.0	50.0	61.8	60.9	64.5	70.0	75.5	90.0	100.0

Table 1b. Effect of IMCO-O on survival of nitrosomonas.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.0	50.0	53.6	51.8	48.2	45.5	38.2	31.8	27.2	19.1
0.1	50.0	54.5	53.6	51.8	51.8	48.2	38.2	30.0	22.7	17.2
1	50.0	54.5	54.5	51.8	51.8	50.0	27.2	28.2	26.4	21.8
10	50.0	46.4	40.9	36.4	30.9	18.2	10.0	10.0	10.0	10.0
100	50.0	45.5	36.4	30.9	19.1	11.8	18.0	18.0	0.0	0.0
1000	50.0	44.5	30.9	24.5	15.5	7.2	7.2	0.0	0.0	0.0
Control 2	50.0	50.0	50.0	54.5	60.9	70.0	70.0	75.5	90.0	100.0

Table 1c. Effect of paradril on survival of nitrosomonas.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.0	50.0	51.8	53.6	52.7	48.2	45.5	40.0	33.6	27.2
0.1	50.0	48.2	45.5	45.5	38.2	29.1	20.0	9.0	4.5	4.5
1	50.0	50.0	50.0	50.0	40.9	43.6	22.7	90.0	0.0	0.0
10	50.0	50.0	50.0	44.5	33.6	18.2	14.5	14.5	0.0	0.0
100	50.0	47.2	40.9	29.1	23.6	16.4	5.4	5.4	0.0	0.0
1000	50.0	47.2	37.2	26.4	17.2	10.0	4.5	4.5	0.0	0.0
Control 2	50.0	50.0	50.0	54.5	60.9	64.5	70.0	75.5	90.0	100.0

Table 1d. Effect of Gel/KCl/Polymer on survival of nitrosomonas.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.0	49.1	45.5	45.5	44.5	36.4	27.2	27.2	20.0	16.4
0.1	50.0	46.4	45.5	45.5	48.2	40.9	36.4	31.8	25.4	19.1
1	50.0	49.1	49.1	45.5	45.5	40.9	30.0	27.2	19.1	13.6
10	50.0	50.0	49.1	42.7	35.5	36.4	16.4	8.1	8.1	13.6
100	50.0	49.1	49.1	49.1	41.8	22.7	24.5	13.6	9.1	0.0
1000	50.0	46.4	46.4	49.1	36.4	36.4	22.7	10.9	9.1	0.0
Control 2	50.0	50.0	50.0	54.5	60.9	64.5	70.0	75.5	90.0	100.0

cant effect on nitrite accumulation by *Nitrosomonas,* nitrite consumption by *Nitrobacter* and evolution of carbon IV oxide by *E. coli*.

Five logarithmic concentrations of each of the drilling fluids: 0.01, 0.1, 1.0, 10.0 and 100.0 mg/L were prepared using appropriate

broth: Winogradsky phase 1, Winogradsky phase II and lactose broth were used as diluents in tests with *Nitrosomonas, Nitrobacter* and *E. coli*, 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 litre conical flask. For water-soluble drilling fluids the volume was then made up to 1000 ml with diluent (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 100 mg of mud was transferred to a 1 L flask and the volume made up to 1000 mL with diluent.

The same was repeated to achieve 10, 1.0, and 0.01 mg/L by dividing the equivalent volume of 100 mg/L of mud by 10, 100, 1000 and 10,000 respectively and making up the volume to 1000 ml 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 (0.1 ml) of *Nitrosomonas* was added to each of the toxicant concentrations contained in 250 ml Erlenmeyer flask. Nitrite concentration accumulated by *Nitrosomonas* was determined by coupling of diazotised sulfanilic acid with α-napthyl-ethylene-diamine dihydrochloride (NED) (APHA, 1998). Control 1 containing Winogradsky phase 1 broth and organism without toxicant was 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 (0.1 ml) 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 72 h. At exposure time of 0, 2, 3, 4, 8, 12, 24, 36 and 48 h nitrite content was determined as in nitrite accumulation test. Control 2 containing Winogradsky phase II broth and organism without toxicant was treated in the same manner.

Carbon 1v oxide evolution test

The method of Allen et al. (1985) was adapted for the determination of evolution of carbon IV oxide by *E. coli*. The standard inoculum (0.1 ml) or *E.* coli was inoculated into each 250 ml 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 250 ml Erlenmeyer flask containing 200 ml of 0.01 M calcium hydroxide solutions. Incubation followed immediately at 37°C for 48 h. At exposure time of 0, 1, 2, 3, 4, 8, 12, 24 and 48 h, 10 ml of Ca $(OH)_2$ were collected from each of the receiving flasks and 0.02 m tetraoxosulphate IV acid (H_2SO_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 22.4 dm^3. The stoichiometric equation of the reaction is given below:

$$Ca(OH)_2(aq) + 2CO_2 \longrightarrow Ca(HCO_3)_2(aq)$$
$$Ca(HCO_3)_2(aq) + H_2SO_4(aq) \longrightarrow CaSO4(s) + 2H_2O + CO_2(g)$$

From step II 1 mole of CO_2 = 1 mole of $CaHCO_3)_2$

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

Where
C_A = Concentration of acid 0.02
V_A = Volume of acid used in titration
V_B = Volume of base used = 10 ml
Mc = moles of CO_2
Actual volume of CO_2 = Mc x 22.4 dm^3

Control 3 contained standard inoculum (0.1 ml) of *E. coli* inoculated into a 250 ml Erlenmeyer flask containing sterile lactose broth with no toxicant.

Percentage survival (mortality) 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 5 ml of broth culture (Standard inoculum) 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 for monitoring enzyme-mediated activities. Plates were incubated at room temperature and those containing between 30 and 300 colonies were selected and counted 48 h later. The percentage survival was calculated by dividing the count in each toxicant concentration by the count in the appropriate control (control 1, 2 or 3) at 48 h incubation and multiplying by 100.

RESULTS

There was a decrease in the percentage survival of *Nitrosomonas* with increasing contact time (exposure period) and concentration of drilling fluid (Tables 1a – 1d). Mortality was greatest at high concentrations (10.0-1000 mg/l) of fluids and at 36 and 48 h exposure period for all systems. IMCO-O concentrations of 0.01,0.1 and 1.0 were stimulatory even at 3 h. Paradril was stimulatory at 0.01 at 4 h while IMCO-W and Gel/KCL/Polymer were toxic at all concentrations and exposure periods. Control 1 showed an increase in the population of viable cells (absence of mortality) with increase in exposure time.

In percentage survival tests involving *Nitrobacter*, (Tables 2a - 2d) IMCO-W was stimulatory at 0.01 mg/l for 8 h; however, other concentrations (1.0-1000 mg/l) were inhibitory through out the exposure periods. Paradril was stimulatory at 0.01 and 0.1 mg/l at 3 h exposure period. Gel/KCL/Polymer was stimulatory at 0.01 mg/l at 36 h exposure period. It was stimulatory at 0.1 mg/l up to 8 h while it was stimulatory at 1.0 and 10.0 mg/l at 3 and 1 h exposure period, respectively. All concentrations of IMCO-O were inhibitory through out all exposure periods. Control 2 showed an increase in the population of viable cells (absence of mortality) with increase in exposure time. The effect of the fluids on the percentage survival of *E. coli* (Tables 3a - 3d) revealed a similar trend to that of *Nitrosomonas* and *Nitrobacter*. Some concentrations were stimulatory while some were inhibitory at different lengths of exposure period. IMCO-W was stimulatory at 0.01 and 0.1 mg/l for 48 and 12 h exposure period, respectively. IMCO-O was stimulatory at 0.01 and 0.1 mg/l at 4 h exposure period. Gel/KCL/Polymer was stimulatory at 0.01 mg/l for 12 h and at 0.1 and 1.0 mg/l at 4 h. Paradril did not show any stimulatory effect at any concentration through out all exposure periods. A decrease in the percentage survival of the organism with

Table 2a. Effect of IMCO-W on survival of nitrobacter.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.5	53.6	52.5	58.7	54.6	54.6	48.4	42.6	42.6	35.1
0.1	50.5	54.6	49.5	34.0	39.1	32.9	28.8	20.6	15.4	9.2
1	50.5	50.5	23.7	14.4	21.6	13.4	17.8	17.8	11.3	6.2
10	50.5	47.4	19.5	9.2	8.2	8.2	4.1	2.1	2.1	0.0
100	50.5	49.5	21.6	9.2	8.3	8.2	3.1	3.1	0.0	0.0
1000	50.5	49.5	11.3	10.3	9.3	8.2	2.1	2.1	0.0	0.0
Control 2	50.5	54.6	54.6	58.7	60.8	67.0	74.6	85.6	93.8	100.0

Table 2b. Effect of IMCO-O on survival of nitrobacter.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.5	48.4	24.7	17.5	10.3	7.2	5.1	5.1	3.2	3.2
0.1	50.5	46.3	17.5	17.5	10.3	6.1	3.2	2.2	2.2	2.2
1	50.5	48.4	11.3	12.3	10.3	6.1	3.2	3.2	3.2	3.2
10	50.5	43.2	15.4	18.5	12.3	8.2	17.5	3.2	3.2	3.2
100	50.5	42.3	12.3	7.2	7.2	7.2	4.1	0.0	0.0	0.0
1000	50.5	42.3	12.3	13.4	7.2	6.1	2.2	0.0	0.0	0.0
Control 2	50.5	54.6	54.6	58.7	60.8	67.0	74.2	87.6	93.8	100.0

Table 2c. Effect of Paradril on survival of nitrobacter.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.5	50.5	52.5	51.5	49.4	42.3	38.1	30.9	28.8	24.7
0.1	50.5	54.6	56.7	54.6	54.6	46.3	34.0	31.9	21.6	19.6
1	50.5	56.7	54.6	50.5	41.2	40.2	26.8	20.6	13.4	9.2
10	50.5	48.4	41.2	41.2	31.9	28.8	17.5	11.3	11.3	10.3
100	50.5	46.3	38.1	31.9	25.7	13.4	4.1	3.1	2.1	2.1
1000	50.5	42.3	36.1	27.8	20.6	13.4	4.1	2.1	2.1	0.0
Control 2	50.5	54.6	54.6	58.7	60.8	67.0	74.2	87.6	93.8	100.0

Table 2d. Effect of Gel/KCl/Polymer on survival of nitrobacter.

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	50.5	54.6	54.6	58.7	60.8	62.8	67.0	77.1	54.6	40.2
0.1	50.5	54.6	54.6	58.7	60.8	58.7	15.5	11.3	11.3	9.2
1	50.5	54.6	54.6	51.5	45.3	38.1	19.6	7.2	6.1	3.1
10	50.5	54.6	54.6	51.5	42.2	41.2	17.5	11.3	4.1	2.1
100	50.5	51.5	39.1	39.1	36.1	21.6	16.5	5.1	3.1	2.1
1000	50.5	48.4	35.1	38.1	35.1	20.6	51.5	3.1	2.1	2.1
Control 2	50.5	54.6	54.6	58.7	60.8	67.0	74.2	87.6	98.9	100.0

increase in exposure time and concentration of the fluids was the general pattern. As in *Nitrosomonas* and *Nitro bacter* the effect was more evident at high concentrations (10.0, 100 and 1000 mg/l) of the fluids. Control 3 showed

Table 3a. Effect of IMCO-W on survival of *E.coli.*

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	35.2	41.8	41.8	43.5	46.3	46.9	52.5	50.2	45.3	40.7
0.1	35.2	41.3	41.3	42.4	44.1	43.0	39.1	34.0	30.2	24.5
1	35.2	34.6	34.6	26.8	22.3	21.7	17.3	10.6	7.2	7.2
10	35.2	32.4	32.4	25.6	20.6	18.9	13.9	10.6	5.5	0.4
100	35.2	32.4	32.4	24.3	19.5	6.1	5.0	3.3	3.3	2.7
1000	35.2	31.8	31.8	22.2	19.5	6.1	5.0	3.3	3.3	2.7
Control 2	35.2	39.6	39.6	48.6	55.8	64.2	74.8	81.5	88.8	100.0

Table 3b. Effect of IMCO-O on survival of *E.coli.*

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	35.2	37.9	43.0	43.0	48.0	31.2	31.2	27.9	22.9	22.3
0.1	35.2	37.9	41.3	36.3	37.9	31.2	28.4	25.1	22.3	17.8
1	35.2	27.9	27.3	25.6	27.9	22.9	21.2	16.7	11.7	11.7
10	35.2	24.5	20.1	20.1	15.6	13.4	11.2	7.8	7.8	6.1
100	35.2	21.7	20.1	18.4	11.2	10.1	8.9	6.7	6.7	5.0
1000	35.2	21.7	21.7	16.7	8.9	8.9	8.9	6.7	6.7	5.0
Control 2	35.2	39.6	45.8	48.6	64.2	64.2	74.8	81.5	77.6	100.0

Table 3c. Effect of Paradril on survival of *E. coli.*

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	35.2	34.0	18.9	19.5	19.5	49.2	39.1	22.3	22.3	22.3
0.1	35.2	22.9	21.7	21.7	21.7	22.9	25.6	18.9	16.7	11.7
1	35.2	17.8	16.2	16.7	18.4	20.6	21.7	16.7	16.2	15.1
10	35.2	16.2	16.2	7.8	7.8	8.3	10.6	12.8	10.1	5.0
100	35.2	22.9	20.1	7.8	6.1	3.4	3.9	2.7	1.1	1.1
1000	35.2	24.5	27.9	7.8	6.1	2.2	2.2	1.1	0.0	0.0
Control 2	35.2	39.6	45.8	48.6	55.8	64.2	74.8	81.5	77.6	100.0

Table 3d. Effect of Gel/KCl/Polymer on survival of *E. coli.*

Concentration	Time (h)									
	0	1	2	3	4	8	12	24	36	48
0.01	35.2	39.6	45.8	38.8	41.8	35.7	39.1	35.2	35.2	32.9
0.1	35.2	39.6	39.1	38.8	37.9	24.0	25.6	16.7	16.7	16.7
1	35.2	39.6	45.8	38.8	45.3	35.2	39.6	29.6	29.6	19.5
10	35.2	39.6	37.4	32.9	45.3	24.0	17.5	6.7	6.7	0.9
100	35.2	37.4	33.5	30.7	18.9	12.8	15.6	5.5	5.5	4.5
1000	35.2	35.1	38.8	25.1	18.9	13.4	15.1	4.5	4.5	3.3
Control 2	35.2	39.6	45.8	48.6	55.8	64.2	74.8	88.8	88.8	100.0

an increase in the population of viable cells (absence of mortality) with increase in exposure time. In Figure 1a-1d nitrite accumulation (oxidation of ammonia to nitrite) by *Nitrosomonas* is presented. In controls nitrite accumula

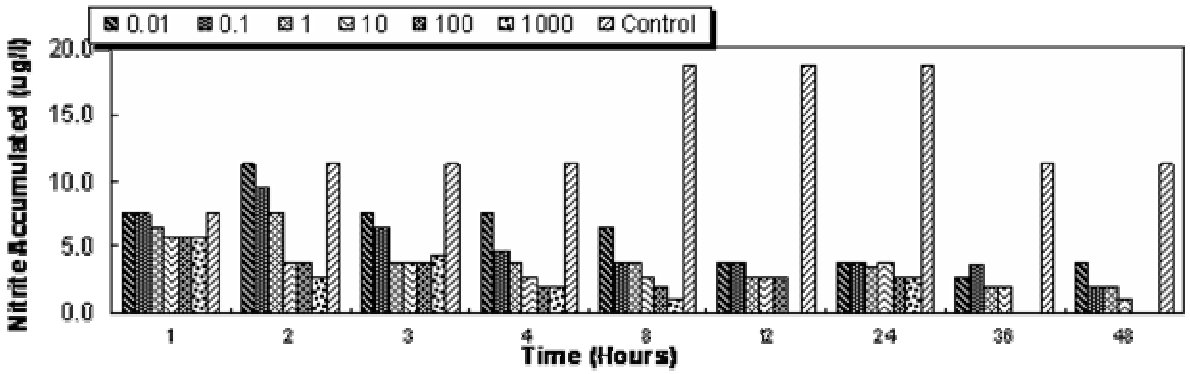

Figure 1a. Effect of paradril on nitrosomonas as a function of nitrite accumulation.

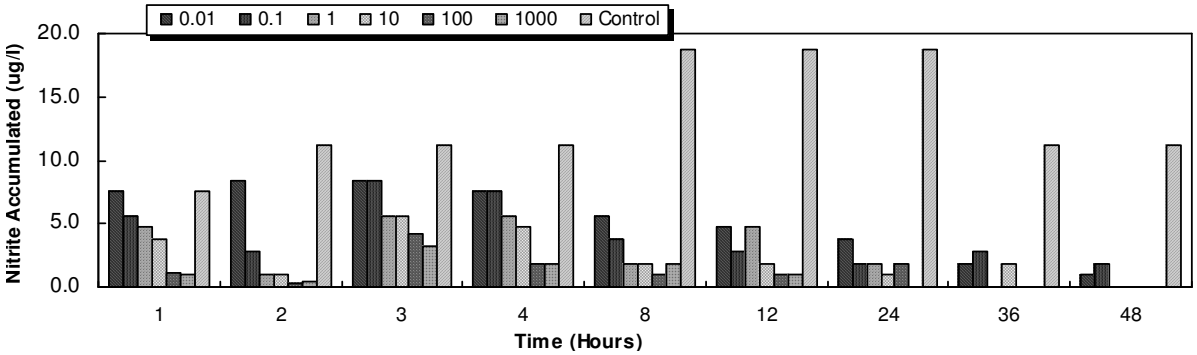

Figure 1b. Effect of IMCO-O on nitrosomonas as a function of nitrite accumulation.

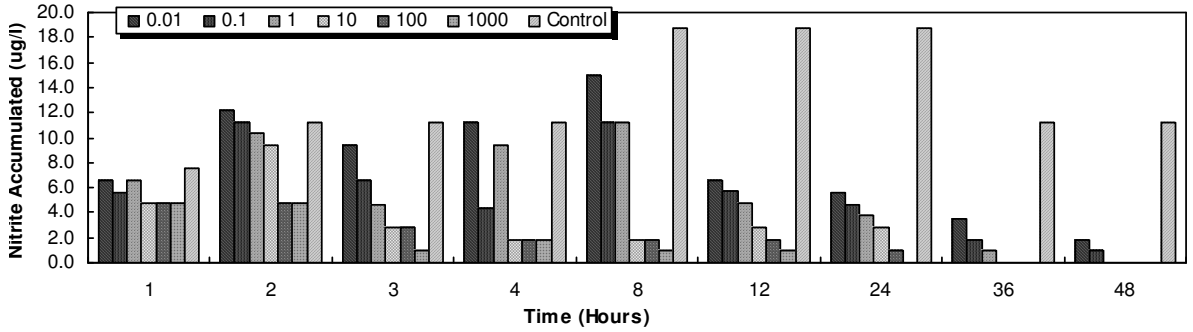

Figure 1c. Effect of IMCO-w on nitrosomonas as a function of nitrite accumulation.

tion increased from 0h to 24 h and decreased to levels similar to the 2, 3 and 4 h during the 36 and 48 h exposure period. Nitrite accumulation by *Nitrosomonas* decreased from 2 to the 48 h exposure period for all toxicant concentration. Results showed that Paradril inhibited nitrite accumulation by Nitrosomonas. A similar effect of IMCO-O (Figure 1b) on *Nitrosomonas* was observed. With IMCO-W the effect on nitrite accumulation by *Nitrosomonas* was not slightly modified. Like the previous two toxicants between 1 and 2 h 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 2 h in IMCO-W there was a slight decrease till 4 h and an increase in the 8 h at lower concentration of 0.01, 0.1 and 1.0 mg/l which was followed by a decrease till the 48 h. 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 expo-

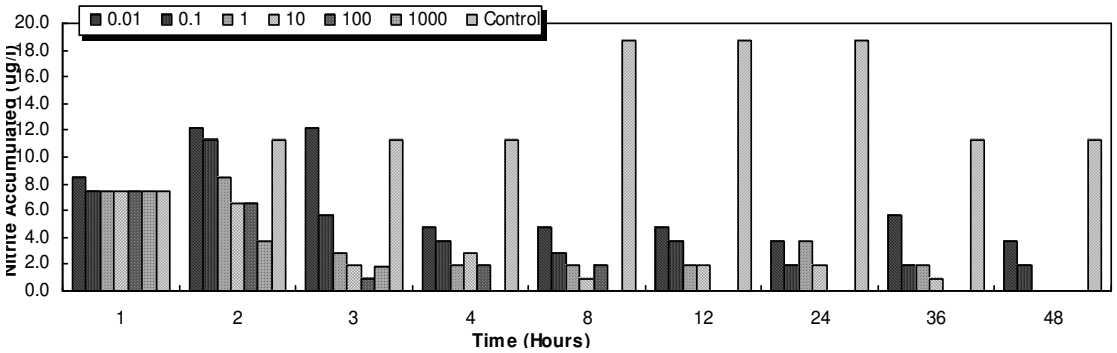

Figure 1d. Effect of Gel/KCL/Polymer on nitrosomonas as a function of nitrite accumulation.

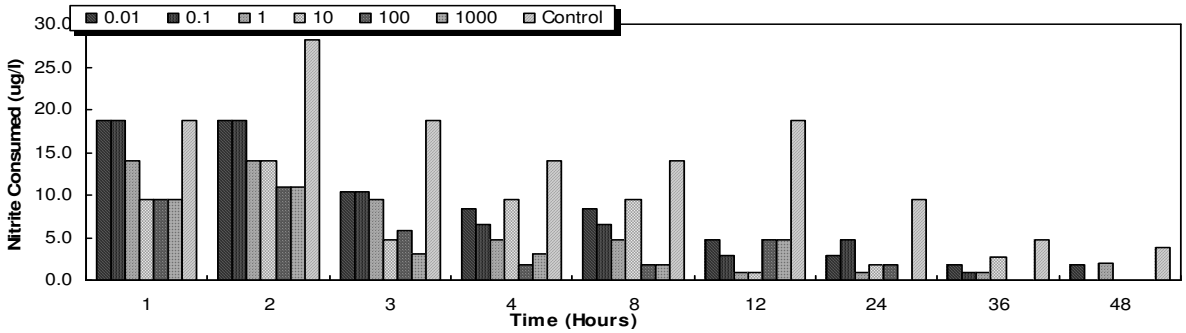

Figure 2a. Effect of IMCO-W on nitrobacter as a function of nitrite consumption.

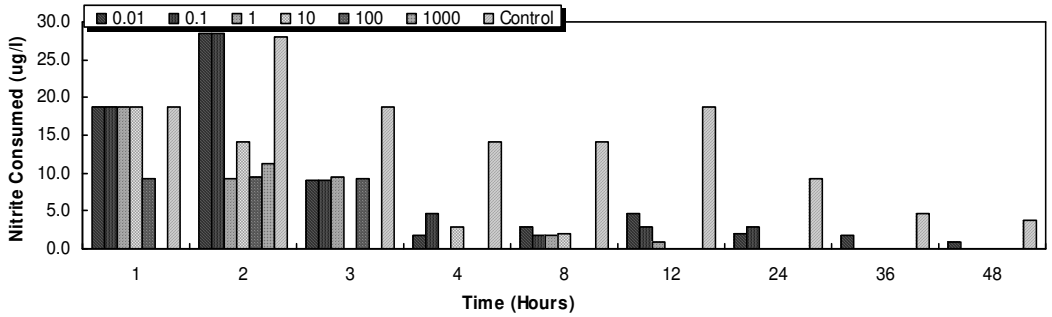

Figure 2b. Effect of IMCO-O on nitrobacter as a function of nitrite consumption.

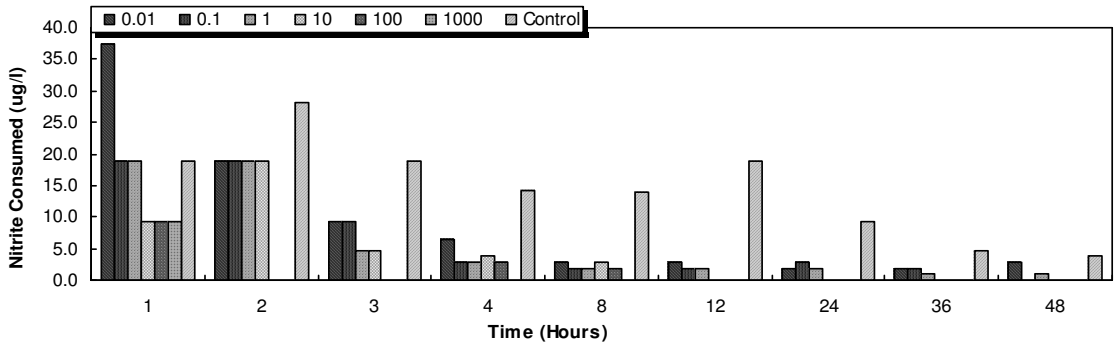

Figure 2c. Effect of paradril on nitrobacter as a function of nitrite consumptio

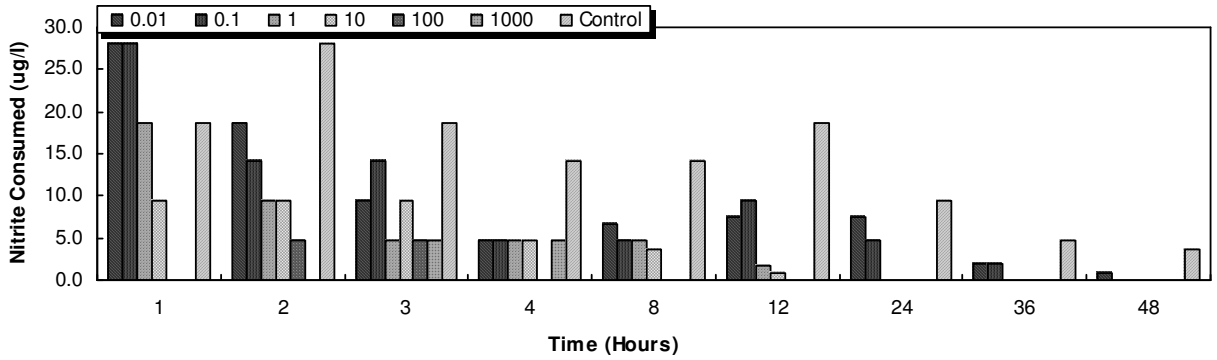

Figure 2d. Effect of paradril on nitrobacter as a function of nitrite consumption.

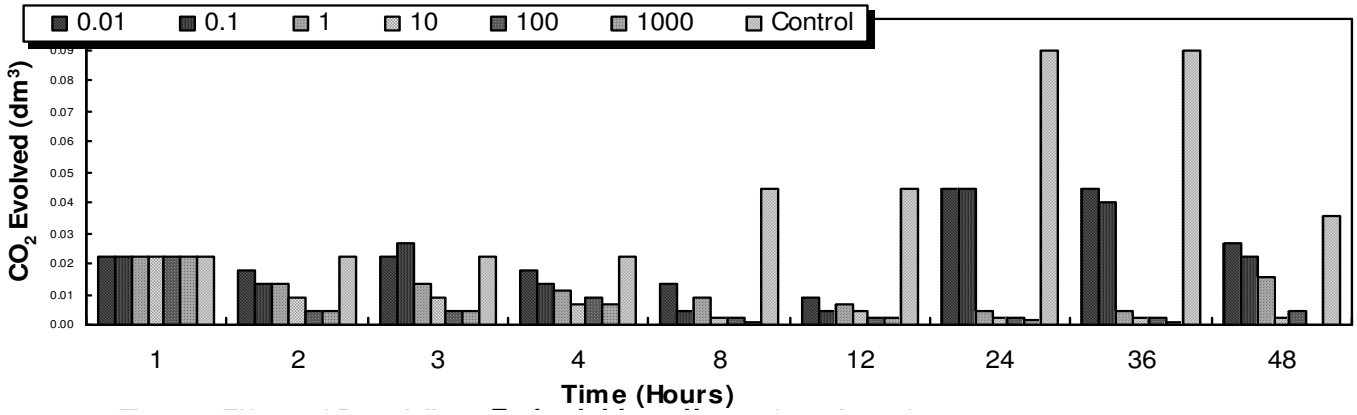

Figure 3a. Effect of paradril on *Escherichia coli* as a function of CO_2 evolution.

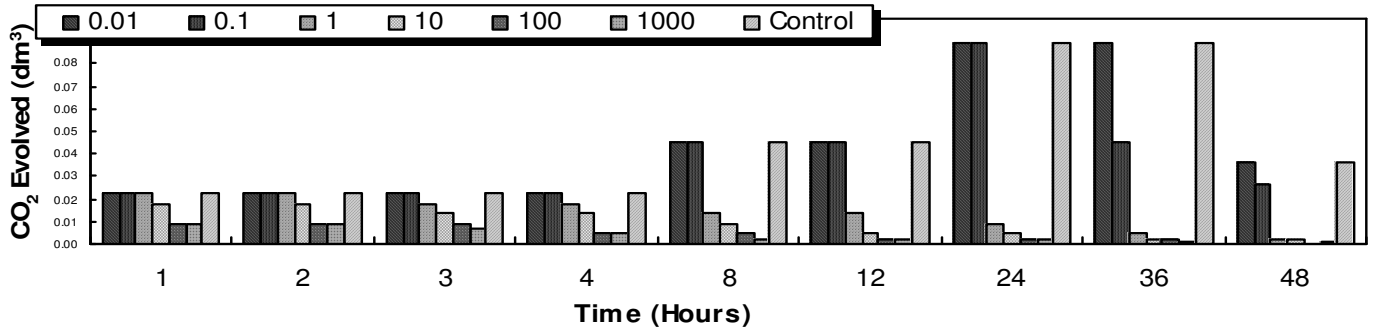

Figure 3b. Effect of Gel/KCL/Polymer on *Escherichia coli* as a function of CO_2 evolution.

sure periods till 48 h especially after the 2 h indicated that nitrite accumulation by *Nitrosomonas* was inhibited with time by the four toxicants.

In Figure 2a - 2d 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 2 to the 48 h exposure period.

However values ranged from 28 µg/l at 2 h to 3 µg/l by the 48 h. Between 3 and 12 h exposure period nitrite consumed during control ranged between 14 to 18 µg/l. Nitrite consumed decreased with time at all concentrations when *Nitrobacter* was exposed to IMCO-W (Figure 2a). 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. In Figure 3a -3d the effect

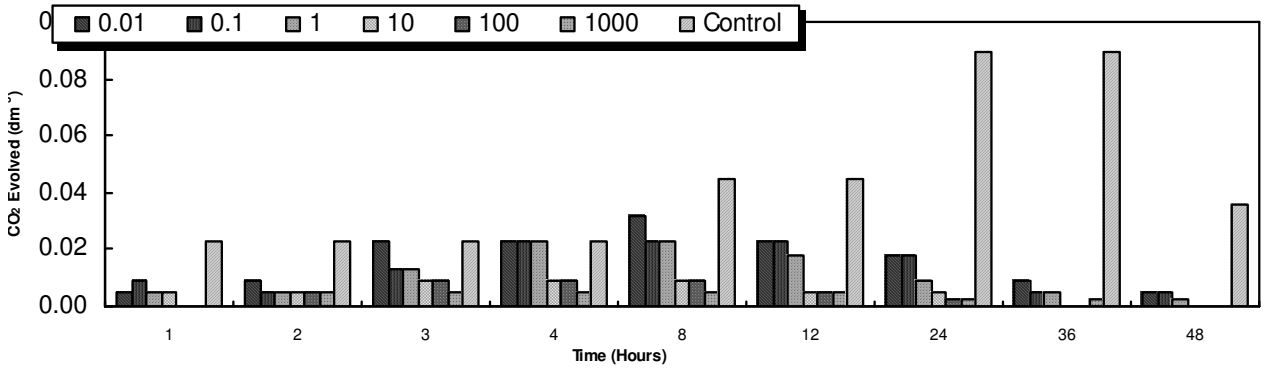

Figure 3c. Effect of IMCO-O on *Escherichia coli* as a function of CO_2 evolution.

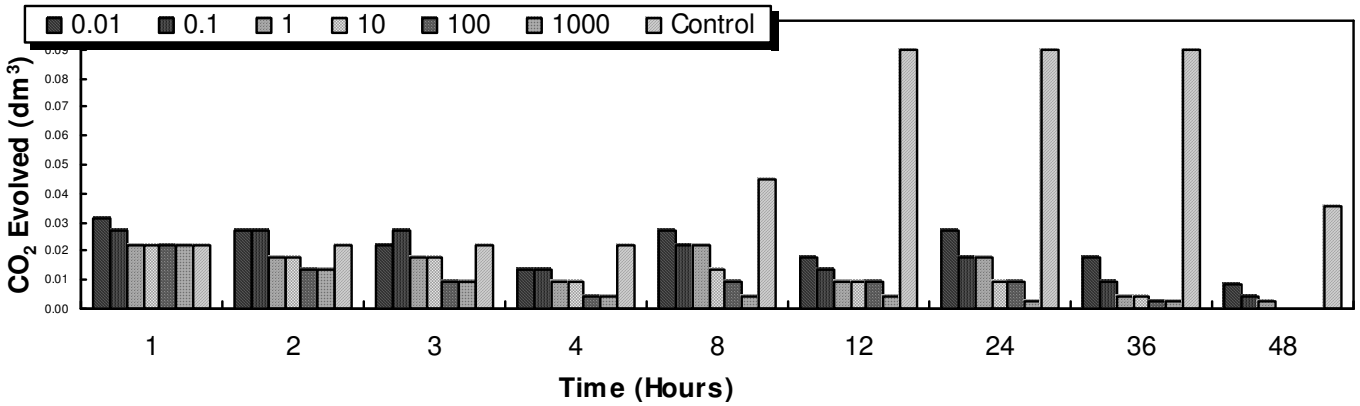

Figure 3d. Effect of IMCO-W on *Escherichia coli* as a function of CO_2 evolution.

on the toxicants on carbon IV dioxide evolution by *E. coli* are presented. Results showed that in the control there was an increase in the amount of $C0_2$ evolved with exposure time. This was evident in the 8 to the 36 h. By the 48 h $C0_2$ evolution decreased. Between 1 and 4 h, $C0_2$ evolution in the control cultures was constant at 0.02 dm^3.

This result showed that there was a lag period of about 6 h before *E. coli* became fully adapted to the environment and started respiring normally resulting in an increase in the $C0_2$ produced. In Figure 3a, $C0_2$ 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 36 h $C0_2$ 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. Lower concentrations of Paradril 0.01 and 0.1 mg/l seemed to stimulate respiration after 24 to 36 h 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 $C0_2$ evolution decreased with time throughout the 48 h exposure period. However at concentration of 0.01 and 0.1 mg/l $C0_2$ evolution increased at the 8 h (0.045 dm^3) and reached its peak at the 24 and 36 h (0.09 dm^3) then declined at the 48 h (0.035 dm^3). These results were identical to that of the control cultures indicating that these concentrations of toxicant were stimulation to $C0_2$ evolution by *E. coli*.

The effect of IMCO-O (Figure 3c) on $C0_2$ evolution by *E. coli* was not exactly similar to that of the other two toxicants. There was a slight increase in $C0_2$ evolution with time (1 to 3 h) from less than 0.01 to 0.02 dm^3. This value was maintained till the 8 h then it reduced from this time to the 48 h. When comparing these values with those of the control $C0_2$ evolution was very much inhibited by this toxicant. There was a decrease in $C0_2$ evolution with time when *E. coli* was exposed to IMCO-W. However the rate of decrease was not as much as that of Paradril.

DISCUSSION AND CONCLUSION

The toxicity of the fluids tested depended partly on their water solubility, chemical composition, concentration (Okpokwasili and Odokuma (1996a) and genetic constitution of the organism (Dutton et al., 1990). For instance

IMCO-O is a highly viscous brown liquid with limited water solubility consisting of barite, calcium hydroxide, sodium hydroxide, sodium chromate, blended fatty acid sulfonates, asphaltic materials and polycyclic aromatic hydrocarbons. The calcium hydroxide, sodium hydroxide and calcium carbonate components of this mixture may increase the pH of water to 14. Limited water solubility or total aqueous insolubility of chemicals increases their toxicity. IMCO-W is water based highly viscous grey liquid also with limited water solubility. Its main components are barite, bentonite asbestos fibre, caustic soda, sodium chromate, calcium hydroxide and soda ash. Because of its similarity in composition with IMCO-O its action may be similar to IMCO-O. Paradril is a viscous reddish brown liquid consisting of barite, bentonite organophilic lignite soluble surfactant carbamate and quarternary amines. The amines increase the pH of the fluid thereby increasing the pH of its environment. Gel/KCl/Polymer is water based highly viscous liquid with limited water solubility. It consists of barite bentonite, sepiolite, carboxylmethyl cellulose polymers, potassium chloride and caustic soda. The presence of sodium hydroxide can increase pH and thereby reduce acidity. Oxidation of ammonia to nitrites by *Nitrosomonas*), nitrites to nitrates, by *Nitrobacter* and carbon (IV) oxide evolution (respiration) by *E. coli* are mediated by enzymes located within the cell membrane of these organisms respectively. These enzymes (proteins) may be denatured by high pH values of these drilling fluids. Chromium in its hexavalent form is toxic to these organisms. Quatenary amines are biocidal, a function of their high pH and the toxicity of the amino functional group. Soluble surfactants present in some of these fluids may dissolve out lipid components of cell membranes, which results in the leakage of cell contents (Nester et al., 1998). The results in Tables 1a-3d revealed that composition and concentration of fluids influenced the various responses of the bacteria. Some concentrations (0.01, 0.1 and 1.0 mg/l) of various fluids were stimulatory to some organisms while other fluids displayed decreases in growth at all test concentrations. Similar, results have been obtained by Okpokwasili and Odokuma (1996). They observed that certain concentrations (10 mg/L) of Carbotrol A9, Chaux lime and Huile clean stimulated the growth of *Nitrobacter* as exposure time increased from 0-4 h.

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 *E. coli* indicating that the bacteria were tolerant to the oil spill dispersants. Okpokwasili and Odokuma (1997) suggested that the toxicity of the dispersants to *Nitrobacter* might 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. All three bacteria exhibited this effect.

Decreases in some metabolic activities of the bacteria (ammonia oxidation by *Nitrosomonas*, nitrite oxidation by *Nitrobacter* and respiration by *E. coli*) were the toxic effects displayed by the drilling fluids. The decrease in nitrite consumption by *Nitrobacter* 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 al., 1997). The membrane being the site of the nitritase enzyme complex mediating respiration (Madigan et al., 1997) thus their inhibition affected the respiration process. Though *Nitrobacter* and *Nitrosomonas* 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 al., 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 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. coli* by the fluids was due to prevention of the fermentation of lactose present in the lactose broth (Dutton et al., 1990). Water solubility, composition and concentration of drilling fluids have been shown to influence the response of nitrifying bacteria and facultative anaerobes such as *E.coli* to these fluids in environments where they are employed. Drilling fluids inhibited two stages of nitrification (ammonia oxidation and nitrite oxidation) by *Nitrosomonas* and *Nitrobacter* respectively. The fluids also inhibited aerobic respiration in *E. coli.* High concentrations of the fluids decreased the growth of all the test organisms. However, lower concentrations were stimulatory in some instances. High and persistent concentrations of these fluids resulting from improper disposal of drill cuttings and fluids may result in the reduction of primary productivity of affected eco-systems due to dislocation in the nitrogen cycle and food chains where aerobic and facultative anaerobic bacteria such as nitrifying and denitrifying organisms feature prominently.

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