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The effect of composition on the biodegradability and toxicity of drilling muds used at ologbo active onshore field, Edo State, Nigeria

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Abstract

The effect of composition on the biodegradability and toxicity of two muds commonly used at onshore oil fields at Ologbo, Edo State were examined. Biodegradation of drill muds by two bacterial and fungal isolates; *Enterobacter aerogenes*, *Micrococcus* sp., *Aspergillus* sp. and *Penicillium* sp. were carried in a shake flask experiment using mineral salts medium at 120 rpm for 28 days. The total viable counts were monitored and ultimate biodegradability was derived from the ratio of COD and BOD₅, after every four days. Lethal effects of the drilling muds on juvenile *Tilapia guineensis*, *Micrococcus* sp. and *Penicillium* sp. were investigated using static renewal bioassay for 96 hr and 24 hr. The Potassium chloride (KCl) polymer water based mud (WBM) was more biodegradable than synthetic based mud (SBM). This was indicated by the highest total viable counts recorded in consortium amended with water based mud (101×10^3 cfu/ml), and also recording the lowest chemical oxygen demand and biological oxygen demand (47 mg/l and 0.4 mg/l respectively). There were no significant differences ($P > 0.05$) in the degradation of the muds by the isolates. The 96 hr LC₅₀ of potassium chloride (KCl) polymer water based mud and synthetic based mud (SBM) were 8125 mg/l and 5800 mg/l for *Tilapia guineensis* respectively. The 24 hr LC₅₀ of Potassium Chloride polymer water based mud was 200 mg/l for *Micrococcus* sp. and *Penicillium* sp. Exploration and production companies operating in Ologbo should be encouraged to put into consideration the effect composition of the drilling muds before usage.

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Introduction

The composition of drilling mud depends upon the requirements of the particular drilling operation. Holes must be drilled through different types of formation requiring different types of drilling fluids (muds). According to Odokuma and Akpanah (2008), in Nigeria, drilling muds 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). The three basic types of drilling muds are water based mud, oil based mud and synthetic based mud (Okoro, 2011). Drilling muds are suspension of solids in liquid emulsion and/or dissolved materials with chemical additives which are employed during exploration to remove cuttings (Vincent-Akpu *et al.*, 2010), and to achieve proper density, viscosity and lubrication characteristics (DPR, 2002, Odokuma and Ikpe, 2003).

Research have abundantly shown that drilling muds additives may contain toxic substances such as heavy metals, hydrocarbons, biocides, chromate, organic polymers and trace elements that have the tendency to bioaccumulate and interfere with normal biological activities of organisms (Odokuma and Ikpe, 2003; Odokuma and Akpanah, 2008; Vincent-Akpu *et al.*, 2010). Several studies according to Engelhard *et al.*, (1989) and Vincent-Akpu *et al.*, (2010), have been conducted with various drilling fluids in the North Sea using mortality as the criterion for determining their effects on the biota. Odokuma and Ikpe (2003) had observed that water based muds were more biodegradable than oil based muds. They ascribed this observation to the greater toxicity of oil based muds. In this study, the effect of concentration on the biodegradability and toxicity of water based mud and synthetic based mud used at Ologbo onshore field were examined. Ologbo community is located in Edo State, which is one of the States in the Niger-Delta region of Nigeria. The improper disposal of drilling muds and cuttings have been found to pose a significant stress on the ecosystem of the receiving

environment (Odokuma and Ikpe, 2003). This work therefore assess the effect of composition on the biodegradability and toxicity of the two main classes of drilling muds now commonly employed in drill operations in this part of Niger-Delta region in Nigeria.

Materials and methods

Source of test isolates and collection of drilling muds

The test isolates employed in this study were all isolated from drill cuttings obtained from a land rig situated at Ologbo Community in Edo State (Imarhiagbe, 2012). Media used were Nutrient agar and Potato Dextrose agar. The various isolates were characterized and identified (Buchanan and Gibbons, 1974, Barnett and Hunter, 1975; Okpokwasili and Okorie, 1988; Cheesbrough, 2000). The geographic position system (GPS) coordinate of the well was E: 350017.978m, N: 229469.956m. The drilling muds used were collected from Nigerian Petroleum Development Company (NPDC) and were coded as synthetic based mud (SBM) and Potassium chloride (KCl) polymer water based mud (WBM). Samples were transported to the laboratory aseptically for evaluation, in labeled plastic containers.

monitoring the ultimate degradability

Biodegradation of drill muds by microorganisms were carried in a shake flask degradation experiment using mineral salts medium. The mineral salt medium contained per litre the following, $MgSO_4 \cdot 7H_2O$, 0.42 g/l, KCl, 0.30 g/l, KH_2PO_4 , 0.8 g/l, K_2HPO_4 , 1.3 g/l, $NaNO_3$, 0.42 g/l, pH 7.4, agar 15 g/l (Okpokwasili and Okorie, 1988). Two bacterial and fungal isolates; namely *Enterobacter aerogenes*, *Micrococcus* sp., *Aspergillus* sp. and *Penicillium* sp. were selected for this test. One hundred and fifty milliliters (150 ml) of the mineral salt medium was dispensed into five (5) different 250 ml conical flasks in duplicate and 10 ml of each drilling mud was added. Bacterial and fungal inoculants for this experiment was prepared by suspending a loopful of each isolate in 2 ml of mineral salt medium. Each organism was introduced into separate conical flask, while consortium of the bacterial and fungal isolates were transferred into

separate conical flasks. The control conical flask remained un inoculated. All flasks were incubated at room temperature on a rotary shaker operating at 120 rpm for 28 days. The total viable counts were monitored and ultimate biodegradability was derived from the ratio of COD and BOD₅, after every four days.

Toxicity assay

The 96 hr acute toxicity bioassay was carried out using juvenile *Tilapia guineensis* according to APHA (1998) and OECD (1995). Synthetic based mud (SBM) and Potassium chloride (KCl) polymer water based mud (WBM) were separately prepared into six different aquaria while the seventh was used as control, without the test chemical. The different concentrations, 1000 mg/l, 4000 mg/l, 5000mg/l, 6000 mg/l, 8000 mg/l and 10000 mg/l of the test chemicals were prepared. The fishes were distributed randomly in batches of ten per concentration into the seven aquaria. The organisms were not touched with bare hands during selections so as to avoid stress due to handling. The fishes were exposed to an initial period of acclimatization. The experiment was observed hourly for any death. Mortality was recorded after 8, 24, 48, 72 and 96 hr. Method used for 24 hr lethal toxicity assay was adapted from Odokuma and Ikpe (2003) using bacteria (*Micrococcus* sp.) and fungi (*Penicillium* sp.). A loopful of the bacterial and fungal cells were collected from their individual slants and dislodged in 10 ml of normal saline, and then allowed to stand for few hours. An approximate cell dilution was chosen (*Micrococcus* sp was 4.0×10^3 cfu/ml and *Penicillium*

sp was 3.3×10^3 cfu/ml). Thereafter, 10 mg/l, 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l and 250 mg/l concentration of the drilling muds were prepared respectively in 250 ml conical flasks. The control was distilled water. The mixture was vigorously shaken for even mixing. One milliliter of each set-up including control was plated out at 0, 2, 4, 8, 12 and 24 hr at 30 °C to determine the viable cells and to assess toxicity. While the bacterial isolate was plated on nutrient agar, the fungal isolate was cultured on Potato Dextrose agar incorporated with chloramphenicol. At the end of incubation, viable cells were counted and recorded. The lethal concentration (LC_{50/24}) values were extrapolated from the graph of mortality against concentration.

Statistical analysis

The analysis of variance of the viable microbial counts obtained was conducted ($\alpha=0.05$) using statistical software (SPSS 17.0).

Results

Tables 1 - 3(A-B) and Figures 1 - 4 showed the ability of *Enterobacter aerogenes*, *Micrococcus* sp., *Aspergillus* sp., *Penicillium* sp., and their respective consortia to degrade the experimental drill muds. The KCl polymer water based mud showed a higher degree of biodegradation when compared with synthetic based mud. This was indicated as the highest total viable counts was recorded in consortium amended with water based mud (101×10^3 cfu/ml), and also recording the lowest chemical oxygen demand and biological oxygen demand (47 mg/l and 0.4 mg/l respectively).

Table 1a. Total viable counts of bacterial isolates in culture medium amended with drilling mud ($\times 10^3$ cfu/ml)

| Bacterial isolates | initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|--|---------|-----|-------|-----|-------|-----|--------|------|--------|------|--------|------|--------|------|--------|------|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| <i>Enterobacter aerogenes</i> | *3.0 | 3.0 | 3.2 | 3.0 | 3.4 | 3.4 | 44.0 | 12.0 | 48.0 | 40.0 | 49.0 | 38.0 | 48.0 | 21.0 | 21.0 | 15.0 |
| <i>Micrococcus</i> sp. | 3.0 | 2.8 | 3.6 | 2.9 | 4.0 | 3.5 | 49.0 | 28.0 | 52.0 | 46.0 | 60.0 | 47.0 | 62.0 | 47.0 | 58.0 | 30.0 |
| <i>Enterobacter aerogenes</i> + <i>Micrococcus</i> sp. | 2.8 | 3.0 | 3.0 | 3.5 | 3.9 | 4.7 | 89.0 | 55.0 | 92.0 | 61.0 | 99.0 | 64.0 | 101.0 | 62.0 | 101.0 | 60.0 |
| Control ⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Legend: *Over all mean values. +Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based mud.

Table 1b. Total viable counts of fungal isolates in culture medium amended with drilling mud ($\times 10^3$ cfu/ml).

| Fungal isolates | initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|--|---------|-----|-------|-----|-------|-----|--------|-----|--------|-----|--------|-----|--------|-----|--------|-----|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| <i>Aspergillus</i> sp. | *3.0 | 3.0 | 3.4 | 3.1 | 4.1 | 3.4 | 5.2 | 3.8 | 5.8 | 4.4 | 6.5 | 5.1 | 6.6 | 5.1 | 6.6 | 4.9 |
| <i>Penicillium</i> sp. | 2.9 | 3.0 | 4.6 | 4.9 | 4.8 | 5.1 | 5.2 | 5.5 | 7.0 | 5.7 | 7.0 | 6.0 | 7.1 | 6.0 | 7.2 | 5.7 |
| <i>Aspergillus</i> sp. + <i>Penicillium</i> sp. | 3.0 | 3.0 | 6.3 | 6.3 | 7.5 | 7.5 | 8.9 | 8.9 | 9.1 | 9.1 | 9.1 | 9.1 | 9.1 | 9.1 | 7.0 | 7.0 |
| Control ⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Legend: Over all mean values. ⁺Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based Mud.

Table 2a. Chemical oxygen demand of bacterial culture medium amended with drilling mud (mg/l).

| Bacterial isolate | initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|---|---------|-----|-------|-----|-------|-----|--------|-----|--------|-----|--------|-----|--------|-----|--------|-----|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| <i>Enterobacter aerogenes</i> | 64* | 98 | 60 | 96 | 55 | 90 | 50 | 85 | 58 | 80 | 57 | 77 | 55 | 65 | 55 | 59 |
| <i>Micrococcus</i> sp. | 64 | 107 | 62 | 105 | 60 | 104 | 58 | 98 | 56 | 91 | 55 | 86 | 55 | 81 | 52 | 73 |
| <i>Enterobacter aerogenes</i> + <i>Micrococcus</i> sp. | 65 | 125 | 60 | 120 | 56 | 115 | 54 | 109 | 52 | 97 | 49 | 70 | 47 | 67 | 47 | 54 |
| Control ⁺ | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |

Legend: *Overall mean values. ⁺Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based mud.

Table 2b. Biological oxygen demand of bacterial culture medium amended with drilling mud (mg/l).

| Bacterial isolate | initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|---|---------|------|-------|------|-------|-------|--------|------|--------|------|--------|-----|--------|-----|--------|-----|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| <i>Enterobacter aerogenes</i> | *17 | 18.1 | 9.7 | 15 | 9.0 | 14.44 | 5.6 | 9.5 | 2.7 | 5.9 | 1.0 | 1.2 | 0.6 | 0.8 | 0.4 | 0.6 |
| <i>Micrococcus</i> sp. | 17.7 | 22 | 11 | 18.4 | 10 | 17.3 | 5.7 | 14.5 | 2.7 | 10 | 1.1 | 1.4 | 0.8 | 1.0 | 0.4 | 0.6 |
| <i>Enterobacter aerogenes</i> . + <i>Micrococcus</i> sp. | 18 | 22.2 | 15.3 | 18.5 | 14 | 18 | 10.5 | 14.7 | 9.0 | 10.5 | 1.4 | 1.0 | 1.0 | 0.6 | 0.4 | 0.5 |
| Control ⁺ | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 |

Legend: *Over all mean values. ⁺Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based mud.

Table 3a. Chemical Oxygen Demand of fungal culture medium amended with drilling mud (mg/l).

| Fungal isolate | Initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|--|---------|------|-------|------|-------|------|--------|------|--------|------|--------|------|--------|------|--------|------|
| | WB M | SB M | WB M | SB M | WB M | SB M | WB M | SB M | WB M | SB M | WB M | SB M | WB M | SB M | WB M | SB M |
| <i>Aspergillus</i> sp. | *64 | 90 | 60 | 89 | 55 | 86 | 50 | 80 | 58 | 75 | 57 | 72 | 55 | 67 | 55 | 64 |
| <i>Penicillium</i> sp. | 64 | 88 | 62 | 85 | 60 | 78 | 58 | 72 | 56 | 66 | 55 | 57 | 55 | 51 | 52 | 48 |
| <i>Aspergillus</i> sp. + <i>Penicillium</i> sp. | 65 | 88 | 60 | 80 | 56 | 75 | 54 | 70 | 52 | 65 | 49 | 65 | 47 | 60 | 47 | 60 |
| Control ⁺ | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |

Legend: *Over all mean values. ⁺Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based mud.

Table 3b. Biological oxygen demand of fungal culture medium amended with drilling mud (mg/l).

| Fungal isolate | Initial | | Day 4 | | Day 8 | | Day 12 | | Day 16 | | Day 20 | | Day 24 | | Day 28 | |
|--|---------|-------|-------|------|-------|------|--------|------|--------|------|--------|------|--------|-----|--------|------|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| <i>Aspergillus</i> sp. | *20 | 20.9 | 20 | 19.6 | 14 | 18.6 | 8.5 | 12 | 2.0 | 2.5 | 1.74 | 2.1 | 0.8 | 1.1 | 0.4 | 0.14 |
| <i>Penicillium</i> sp. | 15.82 | 17.65 | 13.8 | 16.5 | 8.5 | 16.2 | 6.0 | 8.56 | 2.1 | 2.12 | 1.5 | 2.0 | 0.4 | 0.4 | 0.4 | 0.12 |
| <i>Aspergillus</i> sp. + <i>Penicillium</i> sp. | 15 | 16.8 | 13.6 | 15.4 | 8.0 | 10 | 5.5 | 7.0 | 2.0 | 2.1 | 1.4 | 1.58 | 0.4 | 0.5 | 0.4 | 0.11 |
| Control ⁺ | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

Legend: *Over all mean values. +Control contained no isolate, WBM: Water based Mud, SBM: Synthetic based mud.

Table 4. Lethal Concentration (LC₅₀) of drill muds used in the drilling of new on-shore well.

| Drilling mud type | <i>Micrococcus</i> sp. (24hr LC ₅₀) mg/l | <i>Penicillium</i> sp. (24hr LC ₅₀) mg/l | Tilapia (96hr LC ₅₀) mg/l |
|-------------------|--|--|---------------------------------------|
| KCl Polymer WBM | *200 | 200 | >8125 |
| SBM | 180 | 175 | >5800 |

Legend: *Values represent means of duplicates KCl: Potassium Chloride WBM: Water based Mud, SBM: Synthetic based mud.

Table 5a. Effective time for concentration of drill mud toxicity test on *Micrococcus* sp.

| | 0hr | | 2 | | 4 | | 8 | | 12 | | 24 | | Total Mortality | | % Mortality | | |
|----------|-----|----------------------|-----|---------------------|-----|---------------------|-----|---------------------|-----|---------------------|-----|---------------------|---------------------|----------------------|-------------|-----|---|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 10 mg/l | - | - | - | - | - | 1.0×10 ² | - | 1.0×10 ² | - | 1.0×10 ³ | - | 1.2×10 ² | - | 1.32×10 ³ | - | 33 | |
| 50 mg/l | - | - | - | 1.0×10 ² | - | 1.2×10 ³ | - | 1.8×10 ³ | - | 9.0×10 ² | - | - | - | 4.0×10 ³ | - | 100 | |
| 100 mg/l | - | *5.0×10 ² | - | 2.0×10 ³ | - | 1.5×10 ³ | - | - | - | - | - | - | - | 4.0×10 ³ | - | 100 | |
| 150 mg/l | - | 8.0×10 ² | - | 2.5×10 ³ | - | 7.0×10 ² | - | - | - | - | - | - | - | 4.0×10 ³ | - | 100 | |
| 200 mg/l | - | 1.0×10 ³ | - | 3.0×10 ³ | - | - | - | - | - | 2.0×10 ² | - | 2.0×10 ² | 4.0×10 ³ | 5 | 100 | | |
| 250 mg/l | - | 4.0×10 ³ | - | - | - | - | - | - | - | 5.0×10 ² | - | 5.0×10 ² | 4.0×10 ³ | 12.5 | 100 | | |

Legend : + values are in cfu/ml, An inoculum of 4.0 × 10³ cfu/ml was introduced into each solution; (-) means no mortality; WBM = Water Based Mud; SBM = Synthetic.

The results of the toxic effect of the drilling muds on selected test organisms are presented in Tables 4-5(A-C). Table 4 revealed the lethal concentration (LC₅₀) of the drill muds used at the location. The results showed that the synthetic based mud was more toxic to the test organisms than the KCl polymer water based mud. The 96 hr LC₅₀ of KCl polymer water based mud and synthetic based mud were 8125 mg/l and 5800 mg/l for *Tilapia guineensis* respectively. The 24 hr LC₅₀ of KCl polymer water based mud was 200 mg/l for *Micrococcus* sp. and *Penicillium* sp., while synthetic based mud, was 180mg/l and 175 mg/l for *Micrococcus* sp. and *Penicillium* sp. respectively. The effective dead time (Tables 5 A-C) of

the test organisms at different concentrations of the drilling muds revealed that at concentrations 200 mg/l, and 250 mg/l, death of *Micrococcus* sp. and *Penicillium* sp. occurred within 12 to 24 hr of exposure to KCl polymer water-based mud with mortality rate of 2.0 × 10² cfu/ml, 5.0 × 10² cfu/ml and 1.8 × 10² cfu/ml, 1.0 × 10² cfu/ml respectively; while at concentrations 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l of synthetic based-mud, death occurred within zero hr of exposure. The effective time of *Tilapia guineensis* at varied concentrations of KCl polymer water based mud and synthetic based mud were observed to be 96 hr and 48hr of exposure respectively.

Table 5b. Effective time for concentration of drill muds toxicity test on *Penicillium* sp.

| | 0hr | | 2hr | | 4hr | | 8hr | | 12hr | | 24hr | | Total mortality | | % Mortality | | |
|----------|-----|-----------------------|-----|----------------------|-----|---------------------|-----|----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|-------------|-------|---|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 10 mg/l | - | - | - | - | - | 1.7×10 ² | - | 1.0 ×10 ² | - | 1.0×10 ³ | - | 1.2×10 ³ | - | 2.47×10 ³ | - | 74.85 | |
| 50 mg/l | - | - | - | 1.0×10 ² | - | 2.2×10 ³ | - | 8.0 ×10 ² | - | 2.0×10 ² | - | - | - | 3.3×10 ³ | - | 100 | |
| 100 mg/l | - | +8.0 ×10 ² | - | 1.0 ×10 ³ | - | 1.5×10 ³ | - | - | - | - | - | - | - | 3.3×10 ³ | - | 100 | |
| 150 mg/l | - | 8.0×10 ² | - | 2.5 ×10 ³ | - | - | - | - | - | - | 2.0×10 ² | - | 2.0×19 ² | 3.3×10 ³ | 6.25 | 100 | |
| 200 mg/l | - | 2.9×10 ³ | - | 4.0 ×10 ² | - | - | - | - | 1.0×10 ² | - | 2.0×10 ² | - | 3.0×10 ² | 3.3×10 ³ | 9.1 | 100 | |
| 250 mg/l | - | 3.3×10 ³ | - | - | - | - | - | - | 1.8×10 ² | - | 4.1×10 ² | - | 5.9×10 ² | 3.3×10 ³ | 17.88 | 100 | |

Legend : + values are in cfu/ml, An inoculum of 3.3 × 10³ cfu/ml was introduced into each solution;

(-) means no mortality; WBM = water Based Mud; SBM = Synthetic Based Mud.

Table 5c. Effective time for concentration of drill muds toxicity test on *Tilapia guineensis* (fingerlings)

| | 8hr | | 24hr | | 48hr | | 72hr | | 96hr | | Total mortality | | % Mortality | |
|-----------|-----|-----|------|-----|------|-----|------|-----|------|-----|-----------------|-----|-------------|-----|
| | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM | WBM | SBM |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 1000 mg/l | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 4000mg/l | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 5000mg/l | - | - | - | - | - | - | - | - | 2 | - | 2 | - | 20 | |
| 6000mg/l | - | - | - | - | - | 1 | - | 2 | - | 3 | - | 6 | 60 | |
| 8000mg/l | - | - | - | - | - | 2 | - | 3 | 2 | 3 | 2 | 8 | 80 | |
| 10000mg/l | - | - | - | - | - | 3 | - | 4 | 4 | 3 | 4 | 10 | 100 | |

Legend. Ten (10) fingerlings were introduced into each tank; (-) means no mortality; WBM = water Based Mud; SBM = Synthetic Based Mud.

Discussion

According to Okerentugba and Ezeronye (2003) the isolation of certain oil-degrading micro-organisms in a polluted environment is an indication that these micro-organisms are the active degraders of that environmental pollutant. Alan (2006) has earlier demonstrated that the indigenous aerobic microbial populations have the capability to utilize the synthetic base fluid as their sole carbon and energy sources.

In this study a twenty-eight days monitoring of the biodegradation potential of some micro-organisms (*Enterobacter aerogenes*, *Micrococcus* sp., *Aspergillus* sp. and *Penicillium* sp.) revealed a consistent increase and decrease of the total viable counts (cfu/ml). Total viable bacterial counts were observed to be higher in media containing KCl-polymer water based mud than synthetic based mud (Table 1a). This observation may be due to the fact that water based muds do not contain oil in their liquid phase and as such they are non-toxic and also readily degradable, when compared to synthetic based muds that contain oil in their liquid phase which therefore exact relative toxic effect to organisms (Odokuma and Ikpe 2003; Ayotamuno *et al.*, 2009; Okparanma *et al.*, 2010). The highest total viable counts (cfu/ml) were recorded for the broth batches containing consortium of isolates (*Enterobacter aerogenes* + *Micrococcus* sp in KCl-polymer WBM broth; *Enterobacter aerogenes* +

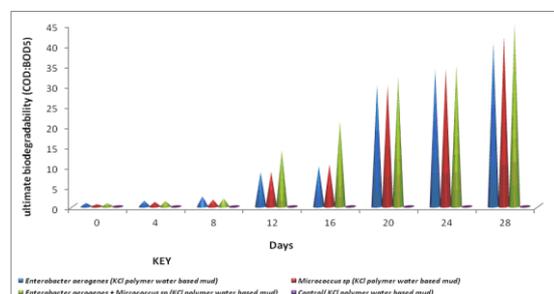


Fig. 1. Percentage ultimate biodegradability of Potassium chloride polymer water based mud by *Enterobacter aerogenes*, *Micrococcus* sp. and a consortium of both isolates.

Micrococcus sp in SBM broth; *Aspergillus* sp + *Penicillium* sp in KCl polymer WBM broth and *Aspergillus* sp + *Penicillium* sp in SBM broth) (Table 1a and 1b). Thus, flasks containing consortium showed marked biodegradation potential in comparison with flasks containing single isolates. Enhanced degradation observed by the microbial consortium in this study may be attributed to the fact that an organism may have acted as primary utilizer, utilizing substrate molecules while the other acted as secondary utilizer, utilizing the breakdown products of substrate after initial attack by primary utilizer (Okpokwasili and Okorie, 1988).

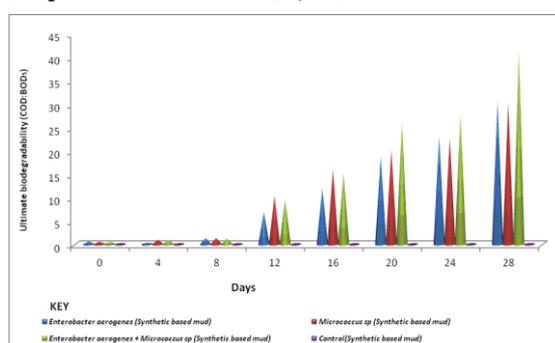


Fig. 2. Percentage ultimate biodegradability of synthetic based mud by *Enterobacter aerogenes*, *Micrococcus* sp. and a consortium of both isolates

Statistical analysis revealed no significant differences ($P > 0.05$) in the degradation of the muds by the isolates. It therefore showed that these selected isolates have potential applications in the bioremediation of sites polluted by water based mud and synthetic based mud.

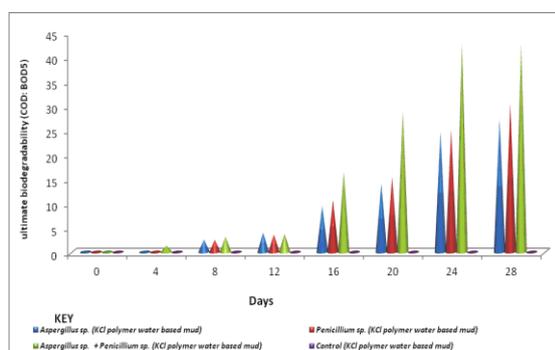


Fig. 3. Percentage ultimate biodegradability of potassium chloride polymer water based mud by *Aspergillus* sp, *Penicillium* sp., and a consortium of both isolates.

The toxicity analysis of the two types of drilling muds with reference to their composition showed that the synthetic based mud was more toxic to the test organisms than the water based mud (Tables 4 5) and may be ascribed to their chemical composition. The increase in percentage mortality as concentration of drilling muds increased over time of exposure in this study is in agreement with previous findings (Ekpo and Ekanem, 2000; Vincent-Akpu, 2010). Vincent-Akpu (2010) had described the positive correlation that exists between toxicant concentrations and mortality coupled with no death in the control tanks against mortality recorded in the treated tanks implied that the drilling mud was responsible for the fish mortality in the respective tanks. Neff *et al.*, (2000), had attributed the toxicity of drilling muds to their hydrocarbon content. The low hydrocarbon content along with other chemical compositions of drilling muds are responsible for their toxicity and also the fact that synthetic based mud does not disperse in water is an additional contributing factor to its toxicity and low biodegradation.

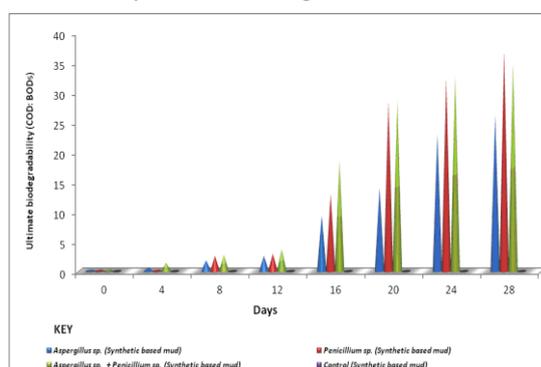


Fig. 4. Percentage ultimate biodegradability of synthetic based mud by *Aspergillus* sp, *Penicillium* sp., and a consortium of both isolates. abbbbbb

Several authors had shown that the toxicity of drilling muds may be linked to their chemical entities such as the base fluid types (Odokuma and Okpokwasili 1992; Ekpo and Ekanem, 2000; Odokuma and Ikpe 2003), concentration, water solubility (Odokuma and Akponah, 2008) and genetic constitution of the organism (Dutton *et al.*, 1990). Therefore, oil exploration and production companies operating in Ologbo community in Edo State, should be encouraged to put into consideration the effect of

chemical composition (nontoxic and ease of biodegradation) of the drilling muds when using these drilling muds.

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