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Evaluation of Microbial Growths on Drilling Muds used in an On-Shore Drilling Well Located in Edo State.

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Abstract:
The growth of indigenous microorganisms on drilling muds used in facilitating a borehole of oil and gas located at Ologbo were evaluated. Growth profiles of bacterial and fungal isolates in mineral salt medium amended with 1% drill muds were examined in a rotary shaker at 120 rpm at 30 ^{0}C for 14 days. The growth kinetics of isolates at different concentrations of drilling muds was assessed (by using the total viable count data to calculate the test organisms' specific growth rate and generation (doubling) time after appropriate incubations). Findings revealed that, the highest bacterial count was 5.8x10^3 cfu/ml recorded for Micrococcus sp. in medium amended with a water based mud and attained its peak at day 6. Contrary, the least fungal count was 0.2x10^3 cfu/ml by Penicillium sp. and Aspergillus sp. in medium amended with synthetic based mud, with a growth peaked at day 8 of incubation. The growth rates (h^{-1}) of the test isolates decrease as concentrations of the drilling muds increased while the generation times were stimulated. The highest growth rate (h^{-1}) of 1.32±0.01 was recorded for Micrococcus sp at 4% and 6% water based mud substrate, while the least growth rate (h^{-1}) of 0.33±0.01 and 0.33±0.05 was recorded for Aspergillus sp at 4% and 6% synthetic based mud substrates respectively. The metabolic abilities of test organisms were clear indications that both synthetic based mud and potassium chloride polymer water based mud are biodegradable and their harmful effects may have been occasioned by oxygen depletion when cuttings are discharged into receiving environment.

Keywords: mud, potassium chloride, isolates, generation, growth rate.

Introduction
The exploration and production industries continue to be faced with the challenges associated with discovering and economically recovering new oil and gas reserves [1]. Explorations of new fields, as well as the exploitation of existing fields, require well drilling operations, along with the continuous task of reducing drilling programme cost intelligent and environmentally prudent disposal solutions for the associated drilling fluids and cuttings. The biodegradation of oil pollutants is not a new concept. It has however, taken a new significance as an increasingly effective and potentially inexpensive clean-up technology [2, 3]. Its potential contribution as counter measure biotechnology for decontamination of oil polluted ecosystems is enormous.

According to the reports of [4, 5], drill cuttings can be defined depending on the drilling muds used to facilitate their drilling process. They are water based mud (KCl polymers or glycerol), oil based mud and synthetic based muds (olefins and esters). Oil based muds have been outlaw by regulatory agencies in the oil producing countries due to the severe adverse environmental effects of oil-based-drill cuttings [1]. Discharge of drilling wastes into the environment without first ascertaining the nil or minimum impacts via carrying out Environmental Impact Assessment (EIA) and Environment Evaluation Report studies (EER) are forbidden [1]. Researches have abundantly shown that naturally occurring microbial degradation mechanisms in the environment result in the bio-destruction of toxic substances such as hydrocarbons and other organic pollutants [6, 7, 8]. The effect of drilling chemicals on nitrate utilization and logarithmic rate of growth of *Nitrobacter* was investigated [9], and the results showed that drilling chemicals inhibit an aspect of nitrification in the biosphere thereby negatively affecting soil and water fertility. Microbial degradation often represents the most desirable form of attenuation because of the irreversible nature of the reaction. In the majority of cases, microbial degradation is a detoxifying mechanism, which leads to complete mineralization [10]. The aim of this study was to evaluate the growths of indigenous microorganisms on the drilling muds used in facilitating the boring of the drill holes under an aerobic condition, with regards to the fate of these muds in the receiving environment.

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Materials and methods

Source of test isolates

Three test isolates (*Enterobacter* sp., *Micrococcus* sp., *Penicillium* sp. and *Aspergillus* sp.,) were employed in this study. They were all isolated from drill cuttings obtained from a land rig situated at Ologbo Community in Edo State [11]. The geographic position system (GPS) coordinate of the well was E: 350017.978m, N: 229469.956m.

Source and collection of drilling muds

The drilling muds used were collected from Nigerian Petroleum Development Company (NPDC) and were coded as synthetic based mud and KCl polymer water based mud. Samples were transported to the laboratory aseptically for microbiological and physico-chemical analyses.

Growth profile of bacterial and fungal isolates on synthetic based mud and potassium chloride polymer water based mud

Four isolates, identified as species of *Enterobacter, Micrococcus, Aspergillus and Penicillium* obtained from the drill mud cuttings were used for the tests. The viability and purity of the different isolates were earlier checked [12]. Two sets of duplicate 250 ml sterile conical flasks, each containing 90ml of the culture medium were prepared. The culture medium used was that described by [13]. The mineral salts medium had the composition of 2.0 g NaCl; 0.42 g $MgSO_4 .7H_2O$; 0.29 g KCl; 0.89 g $KHPO_4$; 1.25 g Na_2HPO_4; 0.42 g $NaNO_3$; 1 litre deionized water. The pH of the medium was 7.4 and bacteriological agar (oxoid) was added at concentration of 1.5% (w/v) and amended with 1% of the drill muds. The bacterial and fungal isolates used were diluted appropriately and 10ml of each of the dilutions were added to the flasks, as inoculums. These set of flasks served as the test flasks while the flasks containing no isolate was used as control. The test and control flasks were incubated under aerobic conditions at 120 rpm at 30 ^{0}C for 14 days. The following parameters were monitored; turbidity (760 nm), total viable counts and pH at 48 hours intervals for 14 days

Growth kinetics of isolates at varied concentrations of drilling muds

The test isolates were streaked onto nutrient agar plates and potato dextrose agar plates from stock culture slants and incubated to check viability and purity. A concentration of 2 %, 4 % and 6 % drill muds ($^v/_v$) were prepared with the mineral salt medium in a 250 ml conical flask, each containing 90 ml of culture medium. Duplicate culture medium was prepared for the appropriate test isolate. The isolates were diluted appropriately and 10ml of each dilution added to the culture medium. The effects of the different concentrations of the drill muds were assessed using the total viable count data to calculate the specific growth rate and generation (doubling) time of the isolates after appropriate incubations [14, 15].

Statistical analysis:

The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and analysis of variance [16].

Result and Discussion

A vast array of micro – organisms (bacteria and fungi) that are capable of enhancing catabolic activities of hydrocarbon pollutants in the environment have been well reported [2, 3, 17, 18].

The growth profile curves of the test isolates are shown in Figures 1 - 4.

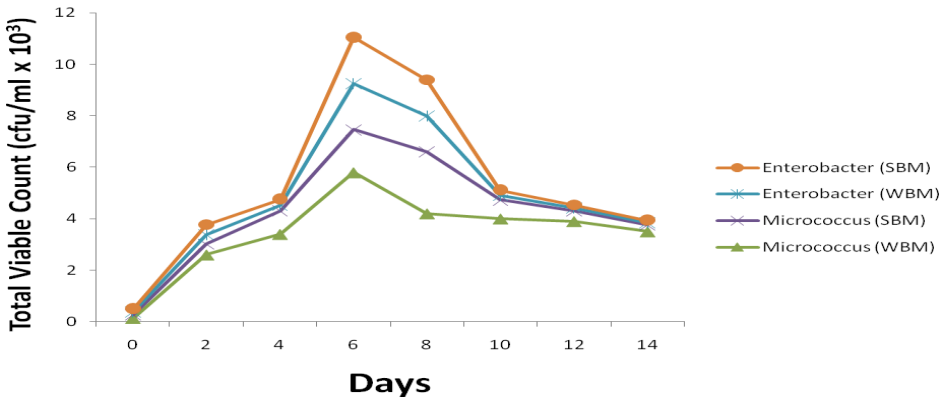


Figure 1: Growth profile of bacterial isolates in culture media amended with synthetic based mud and potassium chloride polymer water based mud.

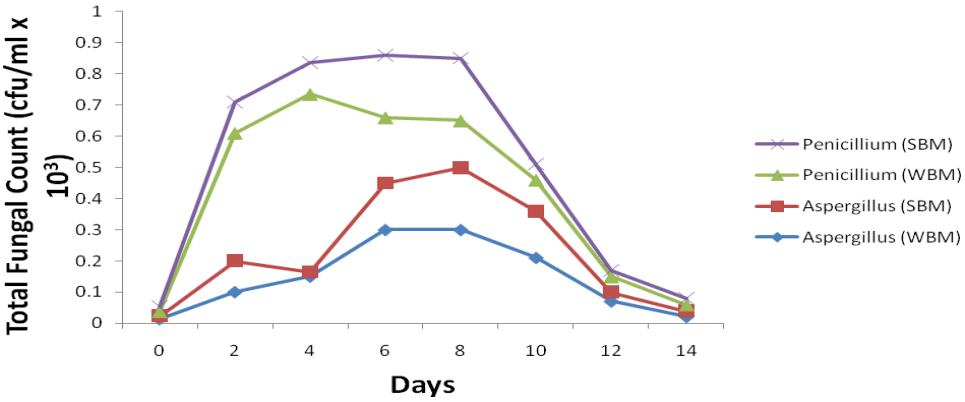
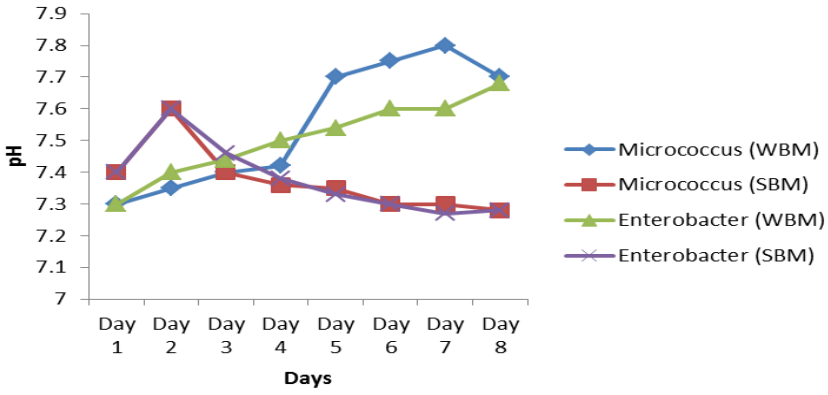


Figure 2: Growth profile of fungal isolates in culture media amended with synthetic based mud and potassium chloride polymer water based mud.

The results obtained showed that the selected isolates strived better in the water based mud (KCl-polymer water based mud) than in synthetic based mud. Considering the chemical composition of these mixtures, this observed growth profiles seem reasonable. The increase in total viable counts and pH revealed the ability of the existing cells to metabolize the drill muds. The observed stepwise curves shown in the figures may have been occasioned by the sequential metabolism of the muds individual congeners and the probable acclimation prior to further degradation of toxic intermediates. However, growths of the selected isolates were faster in medium amended with KCl-polymer water based mud than synthetic based mud. This inference was further depicted by the isolates' total viable counts and growth peaks in the muds. The highest bacterial count of $5.8x10^3$ cfu/ml was recorded for *Micrococcus* sp. in a medium amended with a water based mud and attained its peak at day 6 of incubation, while the least of bacterial count $1.8x10^3$ cfu/ml was recorded for *Enterobacter aerogenes* in medium amended with synthetic based mud, with a growth peaked at day 6 of incubation. However, the highest fungal count of $0.57x10^3$ cfu/ml was recorded for *Penicillium* sp. in a water based mud and attained its peak at day 4 of incubation, while, the least fungal count of $0.2x10^3$ cfu/ml were obtained for *Penicillium* sp. *and Aspergillus* sp. in medium amended with synthetic based mud, with a growth peaked at day 8.



Days

Figure 3: Effect of pH on the growth profile of bacterial isolates in culture media amended with synthetic based mud and potassium chloride polymer water based mud.

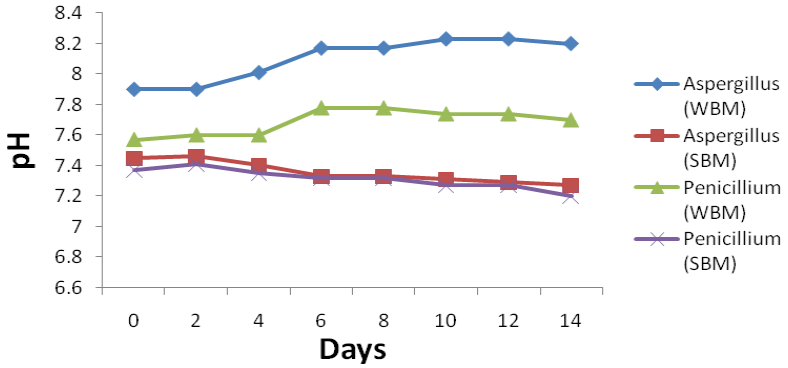


Figure 4: Effect of pH on the growth profile of fungal isolates in culture media amended with synthetic based mud and potassium chloride polymer water based mud.

In this study, it was demonstrated that drill muds inhibit growth rate of isolates with increasing concentration, concomitantly increasing their generation time. However KCl-polymer water based mud showed very slight decreases in the growth rate with increasing concentration. General observation of the growth rates (h^{-1}) (Table 1) of the test isolates (*Enterobacter aerogenes*, *Micrococcus* sp., *Aspergillus* sp. and *Penicillium* sp.) revealed a decreased in their growth rates (h^{-1}) as concentrations of the drilling muds increased.

Table 1: Growth rates of *Enterobacter* sp*; Micrococcus* sp*; Aspergillus* sp. and *Penicillium* sp. grown at varied concentrations of drilling muds.

substrates	Growth rates of (h^{-1}) at different concentrations					Generation times(h) at different concentrations			
	Micro organism	0%	2%	4%	6%	0%	2%	4%	6%
SBM	*Enterobacter aerogenes*	1.0±0.03	1.33±0.01	1.0±0.02	1.0±0.01	1.00±0.02	0.75±0.02	1.00±0.02	1.00±0.04
	Micrococcus sp.	0.7±0.01	1.33±0.03	0.66±0.01	0.33±0.01	1.43±0.01	0.75±0.05	1.52±0.02	3.00±0.01
	Aspergillus sp.	0.66±0.08	0.66±0.01	0.33±0.01	0.33±0.05	1.52±0.01	1.52±0.01	3.00±0.01	3.00±0.04
	Penicillium sp.	0.66±0.03	1.0±0.02	1.0±0.01	0.33±0.01	1.52±0.01	1.00±0.02	1.00±0.01	3.00±0.01
WBM	*Enterobacter aerogenes*	1.0±0.03	1.33±0.02	1.33±0.01	1.0±0.05	1.00±0.02	0.75±0.05	0.75±0.03	1.00±0.01
	Micrococcus sp.	0.7±0.01	1.33±0.07	1.32±0.01	1.32±0.01	1.43±0.01	0.75±0.05	0.75±0.05	0.75±0.01
	Aspergillus sp.	0.66±0.08	1.33±0.04	1.33±0.08	1.0±0.02	1.52±0.01	0.75±0.02	0.75±0.05	1.00±007
	Penicillium sp.	0.66±0.03	1.33±0.02	1.0±0.01	1.0±0.02	1.52±0.01	0.75±0.02	1.00±0.01	1.00±0.01

The highest growth rate (h^{-1}) of 1.32±0.01 was recorded for *Micrococcus* sp in 4% and 6% water based mud substrate, while the least growth rate (h^{-1}) of 0.33±0.01 and 0.33±0.05 was recorded for *Aspergillus* sp in 4% and 6% synthetic based mud substrates respectively. The highest generation time (h) was recorded for isolates cultured in synthetic based mud substrate. Statistical analysis showed no significant difference in the growth profile of the isolates ($P>0.05$) but there was a significant effect of the muds on the selected isolates ($P<0.05$). The metabolic abilities of these organisms are a clear indication that both synthetic based mud and potassium chloride polymer water based mud are biodegradable and their harmful effects may have been occasioned by oxygen depletion when cuttings are discharged into receiving environment [11, 19].

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