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Aerobic Degradation of Drill Muds by Axenic and Mixed Bacterial Isolates from Drill Cuttings at Ologbo, Edo State, Nigeria.

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Abstract

Increasing exploration and production activities, coupled with improper waste disposal practices have encouraged widespread contamination of ecological systems at locations of these activities. This study was to examine the biodegradation potentials of axenic and mixed bacterial isolates associated with drill cuttings emanating from onshore well located at Ologbo, Edo State. Aerobic biodegradation was determined using screen test and shake flask experiment (assessing the total viable counts (cfu/ml), pH, turbidity, Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) for a period of 28 days. Results revealed heavy growth in broth cultures amended with glucose and drill muds. In shake flask experiment, the highest total viable counts of 10.2 x 10^3cfu/ ml and 6.4 x 10^3 cfu/ ml were recorded for cultures containing consortium of isolates (*Enterobacter aerogenes* + *Micrococcus* sp. In water based mud (WBM) broth; *Enterobacter aerogenes* + *Micrococcus* sp. in synthetic based mud (SBM) broth. The COD (reduced from 65 mg/ l at day 1 to 47 mg/ l at day 28) and BOD_5 (reduced from 22.2 mg/ l at day 1 to 0.7 mg/ l at day 28 in synthetic based mud) results are evidence of the oxidation of the substrates. There was no significant difference in the degradation of the drilling muds by the isolates ($p > 0.05$). It was therefore shown that these selected isolates have potential applications in the bioremediation of sites polluted with muds waste.

Keywords: Onshore drill-cutting, drill muds, microorganisms, biodegradation.

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Introduction

Increasing exploration and production (E & P) activities, coupled with improper waste disposal practices have encouraged widespread contamination of both the aquatic and terrestrial ecological systems in the areas of E & P activities (Odokuma &Ikpe, 2003). Contaminants, such as used drilling muds and drill cuttings, are serious threats to the biota of natural ecosystems, especially at the location of the borehole (Okpokwasili & Odokuma, 1990). According to Okoro (2011), three major types of drill muds are water-based-muds, pseudo-oil-based muds (synthetic based muds) and oil-based-muds. In examining the role of composition and toxicity in the degradability of drilling muds, the oil based muds were observed to be relatively toxic to the ecosystems (Odokuma & Ikpe, 2003) and have the most deleterious effect on local environment especially diesel (Okoro, 2011). Enemchukwu and Okpokwasili (2003) have earlier shown that drilling muds additives were biodegradable, when they investigated the biodegradability of drilling mud and some mud additives. The toxic effects of these muds have been found to be either acute or chronic (Okpokwasili & Odokuma, 1996, and Odokuma & Ikpe, 2003). Researches have abundantly shown that naturally occurring microbial degradation mechanisms in the environment result in the biodestruction of toxic substances such as hydrocarbons (Okpokwasili & Amanchukwu, 1988). Microbial degradation often represents the most desirable form of attenuation because of the irreversible nature of the reaction.

Drilling muds are suspensions of solids (e.g clay, barite, small cuttings) in liquid emulsions with chemical additives as required to modify their properties (Ifeadi *et al*., 1985). Drilling requires drilling mud to lubricate the drill bit, carry drill cuttings (rock chippings drilled from the reservoir formation) to the surface and control the down-hole formation pressure of reservoir fluids (Okoro, 2011). This investigation reports the potentials of some indigenous bacteria to biodegrade drilling muds used in exploration in Nigeria.

Materials and methods

Source of test isolates: 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 mineral salts medium. The various isolates were characterized and identified. (Cheesbrough, 2000; Okpokwasili & Okorie, 1988; Buchanan & Gibbons, 1974).
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 evaluation, in labeled plastic containers.

Screening test for drilling muds utilizing microorganisms: The method employed was adopted from Okpokwasili & Okorie, 1988. The mineral salts medium was in volume 9.9 ml and 9.8 ml in separate sets of test tubes. To one set of tubes was added 0.1 ml each of drilling mud only while the other test tube sets were added 0.1ml each of test drill muds and glucose as amendment. All the test tubes were thereafter sterilized by autoclaving at 121^0C for 15 mins, after which they were allowed to cool. On cooling, each set of tube was inoculated with two drops of cell suspension of an isolate in sterile mineral salt broth. A set of control test tubes remained un-inoculated while the other control tubes had no drilling mud and glucose. All test tubes were incubated at 28 ± 2^0C for 14 days, after which each tube was scored for optical density.

Shake flask biodegradation test: One hundred fifty milliliters (150 ml) of the medium was dispensed into seven (7) different 250 ml conical flasks in duplicate and 10 ml of each drilling mud (synthetic based mud and water based mud) was added. Bacterial (*Enterobacter aerogenes, Micrococcus* sp.) inoculants for this experiment were prepared by suspending a loopful of each isolate in 2ml of mineral salt medium. Each organism was introduced into separate conical flask, while consortia of the bacteria were transferred into separate conical flasks. The control conical flask remained uninoculated.
All flasks were incubated at room temperature on a rotary shaker operating at 120 rpm for 28 days. The total viable counts, pH, turbidity; COD and BOD_5 were monitored every four days.

Physico-chemical analysis of drill cuttings: pH determination was by a single electrode pH meter(Jen-way Patterson scientific, London) was employed (APHA,1998).
Turbidity determined using spectrophotometric method at appropriate wave length 750 nm. (APHA, 1998). BOD_5 and COD were determined using oxidation methods (APHA, 1998).

Result and discussion

The screen test showing the utilization of synthetic based mud, potassium chloride polymer water based mud and amendments of individual muds with glucose is shown in Table 1. Results showed heavy growth in broth culture amended with glucose, when compared with media consisting of KCl– polymer water based mud, and synthetic based mud only.

The results on the ability of *Enterobacter aerogenes, Micrococcus* sp. and a consortium of both *Enterobacter aerogenes* and *Micrococcus sp*. to degrade potassium chloride polymer water based mud and synthetic based mud is show on Tables 2 – 4 . The highest total viable counts (10.2 x 10^3cfu/ ml and 6.4 x 10^3 cfu/ ml respectively) were recorded for the broth cultures containing consortium of isolates (*Enterobacter aerogenes* + *Micrococcus* sp. in WBM broth; and *Enterobacter aerogenes* + *Micrococcus* sp. in SBM broth respectively).

Table 1: Screen Test Showing the Utilization of Synthetic Base Mud, Potassium Chloride Polymer-Water Base Mud And Glucose.

Isolate	SBM	KCl polymer WBM	KCl WBM+Glucose	SBM+Glucose
Enterobacter aerogenes	+	++	+++	++
Micrococcus sp.	+++	+++	+++	+++
Bacillus sp.	++	+++	+++	+++
Staphylococcus sp.	+	++	+++	++
Mycobacterium sp.	_	+	++	++
Bacillus sp.	++	+	+++	+++
Enterobacter aerogenes	++	++	+++	++
Pseudomonas sp.	+	+++	+++	+++
Citrobacter freundii	++	+++	+++	++

KEY: +++ Heavy growth; ++ Moderate growth; + Scanty; - No growth

Table 2A: Total Viable Counts of Isolates in Culture Medium Amended with Water Based Mud (10^3 Cfu/Ml)

	Initial	Day4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Enterobacter aerogenes in WBM	2.0	3.2	3.4	4.4	4.8	4.9	4.8	2.1
Micrococcus sp. in WBM	3.0	3.6	4.0	4.9	5.2	6.0	6.2	5.8
Enterobacter aerogenes +*Micrococcus* sp. in WBM	2.8	3.6	4.9	8.9	9.2	9.9	10.1	10.2
Control	0	0	0	0	0	0	0	0

Table 2B: Total Viable Counts of Isolates in Culture Medium Amended with Synthetic Based Mud (10^3 Cfu/Ml)

	Initial	Day4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Enterobacter aerogenes in SBM	3.0	3.0	3.4	3.8	4.0	3.8	2.1	1.5
Micrococcus sp. in SBM	2.8	2.9	3.5	2.8	4.6	4.7	4.7	3.0
Enterobacter aerogenes +*Micrococcus* sp. in SBM	3.0	3.5	4.7	5.5	6.1	6.4	6.2	6.0
Control	0	0	0	0	0	0	0	0

Overall mean value

Table 3A: Biodegradative Potentials of *Enterobacter aerogenes, Micrococcus sp.,* and a Consortium of Both Isolates in Potassium Chloride Polymer Water Based Mud Medium as Shown by pH and Turbidity Values.

	initial		Day 4		Day 8		Day 12		Day 16		Day20		Day 24		Day 28	
	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity
Enterobacter aerogenes	7.18	32	7.35	32	7.43	34	7.7	36	8.05	37	8.07	31	8.1	28	8.11	28
Micrococcus sp	7.13	30	7.31	34	7.44	37	7.73	39	8.18	41	8.22	45	8.25	48	8.28	41
Enterobacter aerogenes +Micrococcus sp	7.14	32	7.45	32	7.47	45	7.69	46	7.85	48	8.15	60	8.2	60	8.24	56
Control	7.02	22	7.01	22	7.02	22	7.0	22	7.02	22	7.02	22	7.02	22	7.02	22

Overall mean value

Table 3B: Biodegradative Potentials of *Enterobacter aerogenes, Micrococcus sp.,* and a Consortium of Both Isolates in Synthetic Based Mud Medium as Shown by pH and Turbidity Values.

	initial		Day 4		Day 8		Day 12		Day 16		Day20		Day 24		Day 28	
	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity
Enterobacter aerogenes	7.18	32	7.35	32	7.43	34	7.7	36	8.05	37	8.07	31	8.1	28	8.11	28
Micrococcus sp	7.13	30	7.31	34	7.44	35	7.73	37	8.18	41	8.22	45	8.25	48	8.28	41
Enterobacter aerogenes +Micrococcus sp	7.14	32	7.45	32	7.47	36	7.69	40	7.85	48	8.15	55	8.2	58	8.24	56
Control	7.02	22	7.01	22	7.02	22	7.0	22	7.02	22	7.02	22	7.02	22	7.02	22

Overall mean value

Table 4A: Biodegradative Potentials of *Enterobacter aerogenes*, *Micrococcus sp.*, and a Consortium of Both Isolates in Potassium Chloride Polymer Water Based Mud Medium as Shown by BOD_5 and COD Values.

	initial		Day 4		Day 8		Day 12		Day 16		Day20		Day 24		Day 28	
	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD
Enterobacter aerogenes	17	64	9.7	60	9	55	5.6	50	2.7	58	1.0	57	0.6	55	0.4	55
Micrococcus sp	17.7	64	11	62	10	60	5.7	58	2.7	56	1.1	55	0.8	55	0.4	52
Enterobacter aerogenes +Micrococcus sp	18	65	15.3	60	14	56	10.5	54	9	52	1.4	49	1.0	47	0.6	47
Control	0.2	40	0.2	40	0.2	40	0.2	40	0.2	40	0.2	40	0.2	40	0.2	40

Overall mean value

Table 4B: Biodegradative Potentials of *Enterobacter aerogenes, Micrococcus sp.*, and a Consortium of Both Isolates in Synthetic Based Mud Medium as Shown by BOD_5 and COD Values.

	initial		Day 4		Day 8		Day 12		Day 16		Day20		Day 24		Day 28	
	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD	BOD_5	COD
Enterobacter aerogenes	18.1	96	15	96	4.44	90	9.5	85	5.9	80	1.2	77	0.8	65	0.6	59
Micrococcus sp	22	107	18.4	105	17.3	104	14.5	98	10	91	1.4	86	1.0	81	0.6	73
Enterobacter aerogenes +Micrococcus sp	22.2	125	18.5	120	18	115	14.7	109	10.5	97	1.4	70	1.1	67	0.7	54
Control	0.3	89	0.3	89	0.3	89	0.3	89	0.3	89	0.3	89	0.3	89	0.3	89

Overall mean value

Discussion

This study has demonstrated that drill cuttings from Ologbo community habour population of microorganisms with the capability to utilize and degrade these drilling muds. Previous investigations have shown that both Gram positive and Gram negative bacteria can enhance catabolic activities of hydrocarbon pollutants in the environment. (Okerentugba and Ezeronye 2003 and Ilori *et al*, 2007). The Gram positive bacterial genera from this investigation included *Micrococcus* sp*, Bacillus* spp*, Staphylococcus* sp.*and Mycobacterium* sp. while the Gram negative bacteria were *Enterobacter aerogenes*, *Pseudomonas* sp. and *Citrobacter freundii*. Earlier investigation by Nnubia and Okpokwasili (1993) indicated that *Staphylococcus* sp and *Bacillus* sp predominated as utilizers among bacteria isolated from drill mud cuttings. The result of screen test revealed that drill muds amended with glucose are more readily utilizable substrate for the growth of microorganisms. Furthermore, KCl – polymer water based mud, also was better utilized than synthetic based mud.

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. The twenty-eight days monitoring of the biodegradation potentials of *Enterobacter aerogenes* and *Micrococcus* sp. revealed a consistent increase and decrease of the total viable counts (cfu/ml). In all tests, total viable counts were observed to be higher in media amended with KCl-polymer water based mud than synthetic based mud. This observation may be due to the fact that water based muds do not contain oil in their liquid phase and such they are non-toxic and also readily degradable, when compared with synthetic based muds that contain oil in their liquid phase therefore exacting toxic effect on organisms (Okparanma *et al*., 2010, Ayotamuno *et al*., 2009; Odokuma and Ikpe 2003). The observance of high counts in tests containing consortiums indicate that high amount of biodegradation of the mud can be achieved by employing culture containing consortium of isolates rather than single isolates.

The pH values of the cultures were within the optimum limits for biodegradation. Mineralization had been reported to be influenced by pH and the optimum pH value required for degradation activity is 6.5-8.0 (Mandrin and Lin, 2007). Carbon removable in a waste water treatment process can be measured by Biological Oxygen demand (BOD) and Chemical Oxygen demand (COD) and these parameters have been known to provide slightly different but complementary information on the carbon in water (Prescott *et al*., 2005). The COD (reduced from 65 mg/ l at day 1 to 47 mg/ l at day 28 in synthetic based mud) and BOD (reduced from 22.2 mg/ l at day 1 to 0.7 mg/ l at day 28 in synthetic based muds) results are evidence of the oxidation of the substrates. Enhanced degradation observed by microbial consortiums may be attributed to the fact that some organisms acted as primary utilizers, utilizing substrate molecules while others acted as secondary utilizers, utilizing the breakdown products of substrate after an initial breakdown by primary utilizers (Atuanya and Purohit, 2001 and Okpokwasili and Okorie, 1988). However, there are reports in the literature that support axenic bacteria being better petroleum degraders compared to mixed bacterial cultures (Odjadjare et al., 2008 and Okerentugba and Ezeronye, 2003). Statistical analysis revealed no significant differences ($P>0.05$) in the degradation of the muds by the isolates.

The findings of this study show that these selected isolates have potential applications in the bioremediation of sites polluted by water based mud and synthetic based mud.

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