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MYCODEGRADATION OF DRILL MUDS BY AXENIC AND MIXED FUNGAL ISOLATES FROM DRILL CUTTINGS AT OLOGBO, EDO STATE, NIGERIA.

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Abstract

Drilling produces large volumes of drilling waste, which can pose significant threats to soil, water quality and development. Fungi are known to degrade a wide variety of materials and compounds. This study examined the mycodegradation potentials of axenic and mixed fungal isolates associated with drill cuttings emanating from onshore well located at Ologbo, Edo State. Aerobic mycodegradation was determined using shake flask experiment (assessing the total viable counts (cfu/ml), pH, turbidity, Biological Oxygen Demand (BOD5) and Chemical Oxygen Demand (COD)were also determined for a period of 28 days. Results of shake flask experiment revealed the highest total viable count (9.1 x 102 cfu/ ml) for the broth cultures containing consortium of isolates on day 16. Generally, the pH and turbidity were in the range of 7.16 to 8.37and 33 to 63 respectively. The reduction in COD (from 88 mg/ l at day 1 to 60 mg/ l at day 28) and BOD5 (from 20.9 mg/ l at day 1 to 0.14 mg/ l at day 28) were evidence of the oxidation of the substrates. The growth profile showed that Aspergillus and Penicillium strived better in the water based mud than in synthetic based mud, with Penicillium sp. having the highest fungal count of (0.57x103 cfu/ml) and attained its peak at day 4.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 mud waste.

Keywords:
Onshore, drill-cutting, drill muds, fungi, mycodegradation.
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1.0 Introduction:

In oil and gas operations, drilling fluids, also referred to as drilling muds, are used to lubricate and cool the drilling apparatus, transport drill cuttings to the surface and seal off porous geologic formations [9, 10, 11, 17, and 20]. Drilling fluids typically consist of bentonite and a range of additives mixed with fresh water or hydrocarbons. The two primary types of drilling muds are water based muds and non-aqueous based muds [14]. Water based muds consist of water mixed with bentonite clay and additives such as barium sulfate (barite), are used for most types of drilling. The non-aqueous drilling muds comprise all non-water and non-dispersible based muds and they include Oil Based Muds (OBM), Low Toxicity Mineral Based Muds (LTMBM), Enhanced Mineral Oil Based Muds (EMOBM) and Synthetic Based Muds (SBM) [14]; and are mostly used in offshore wells or other water sensitive formations. The oil and gas industry has developed technologies and practices to reduce environmental damage with advanced chemical gel drilling fluid systems rather than traditional drilling fluids [11]. Environmental impacts associated with advanced drilling waste disposal are unclear.

Fungi are known to degrade a wide variety of materials and compounds - processes known as mycodegradation. According to Singh 006 [23], fungi have been harnessed by humans in many diverse applications for thousands of years. Fungi are usually slow in growth and often require substrates for cometabolism and their liquid cultures constitute appropriate model systems to explore the biotransformation of a wide variety of compounds [23]. During the last decade, fungi have been used in the treatment of a wide variety of wastes and

wastewaters, and the role of fungi in the bioremediation of various hazardous and toxic compounds in soils and sediments has been established [23]. Fungi have also demonstrated the removal of metals and the degradation and mineralization of phenols and chlorinated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated insecticides and pesticides, dyes, biopolymers, and other substances in various matrices [23]. Polyethylene, with a molecular weight of 4000 to 28 000, is degraded by the cultivation of *Penicillium simplicissimum*YK [24]. Enzymes of *Mucor rouxii*NRRL 1835 and *Aspergillus flavus* have produced changes in the mechanical properties and weight of disposable polyethylene bags [5]. The white-rot fungi are also efficient in polyethylene degradation [12]. *Aspergillus flavus* colonized and degraded chitosan-graft polymethyl methacrylate film by 45% during 25 days of aerobic cultivation in an earlier study [6].

Several authors had shown the ability of bacterial isolates to biodegrade drilling muds [9, 10, 15, 17, 20] but mycodegradation of drilling muds by fungal isolates seems to suffer a great deal of scientific information dearth. The aim of this investigation therefore was to evaluate the mycodegradation potentials of indigenous fungi 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.

2.0 Materials and methods

2.1 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 (N: 229469.956 m E: 350017.978 m) in Edo State [8]. Media used were potatoes dextrose agar and mineral salts medium. The various isolates were characterized and identified [4, 21, 2].

2.2 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.

2.3 Shake flask mycodegradation test:

One hundred fifty millilitres (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. Fungal (*Aspergillus* spp. *and Penicillium* spp.) inoculants for this experiment were prepared by suspending a loopful of each isolate (fungal spores) in 2 ml of mineral salt medium. Each organism was introduced into separate conical flask, while consortia of the fungi 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.

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

Two sets of duplicate 250 ml sterile conical flasks, each containing 90 ml of the culture medium. The culture medium used was that described by [21]. The mineral salts medium had the composition of 2.0g NaCl; 0.42g $MgSO_4 .7H_2O$; 0.29g KCl; 0.89g $KHPO_4$; 1.25g Na_2HPO_4; 0.42g $NaNO_3$; 1 litre deionized water and amended with 2 % of the drill muds. The pH of the medium was 7.4. The fungal isolates (*Aspergillus*sp. And *Penicillium*sp.) 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. Both the test and control flasks were incubated with shaking at 120 rpm at 30^0C for 14 days. The turbidity was read at 450 nm, total viable counts and pH of the pure cultures at 48 hours intervals for 14 days.

2.5 Chemical analysis of drill muds:

pH was determined using single electrode pH meter (Jen-way Patterson scientific, London). Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) were carried out using oxidation methods [1].

3.0 Statistical analysis:

The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and analysis of variance [18].

4.0 Results and discussion

The results on the ability of *Aspergillus* sp., *Penicillium* sp. and a consortium of both *Aspergillus* sp. and *Penicillium* sp. to degrade potassium chloride polymer water based mud and synthetic based mud is show on Tables 1 – 3. The highest total viable counts of 9.1×10^2 cfu/ ml at day 16 and 7.9×10^2cfu/ ml at day 20 were recorded for the broth cultures containing consortium of isolates amended with water based mud; and also with synthetic based mud amendment respectively. Generally, the pH and turbidity of the experiments for broth cultures

amended with water based mud was in the range of 7.16 to 8.37and 33 to 63 respectively. Conversely, broth cultures amended with synthetic based mud had pH and turbidity range of 7.11 to 8.15and 31 to 57 respectively (table 2A and B).The results showed that COD reduced from 88 mg/ L at day 1 to 60 mg/ l at day 28(table3A).It was also observed that BOD_5reduced from 20.9 mg/ L at day 1 to 0.14 mg/ l at day 28 in broth cultures containing consortium amended with synthetic based mud, as evidence of the oxidation of the substrates (table3B).

The growth profile curves of the test isolates are shown in Figures 1-2. The results obtained showed that the two isolates thrived better in the water based mud (KCl-polymer water based mud) than in synthetic based mud. *Penicillium* sp. had the highest fungal count of ($0.57x10^3$ cfu/mL) in a water based mud and attained its peak at day 4. Conversely, the least fungal counts 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.

Table 1A**:** Total Viable Counts of Isolates in Culture Medium Amended with Water Based Mud (10^2 Cfu/mL)

	Initial	Day4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Aspergillus sp. inWBM	3.0	3.4	4.1	5.2	5.8	6.5	2.8	2.1
Penicillium sp. in WBM	2.9	4.6	4.8	5.2	7.0	7.0	4.2	4.2
Aspergillus sp + *Penicillium* sp in WBM	3.0	6.3	7.5	8.9	9.1	8.9	8.8	5.2
Control	0	0	0	0	0	0	0	0

Table 1B**:** Total Viable Counts of Isolates in Culture Medium Amended with Synthetic based MUD (10^2cfu/mL)

	Initial	Day4	Day8	Day12	Day16	Day20	Day 24	Day28
*Aspergillus*sp. inSBM	3.0	3.1	3.4	3.8	4.4	5.1	1.2	1.0
Penicillium sp. in SBM	3.0	4.9	5.1	5.5	5.7	6.0	6.0	3.2
*Aspergillus*sp + *Penicillium*sp in SBM	3.0	6.0	6.0	6.5	7.1	7.9	5.8	5.2
Control	0	0	0	0	0	0	0	0

Overall mean value

Table 2A**:** Biodegradative potentials of *aspergillus* sp., *penicillium* sp., and a consortium of both isolatesin 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
Aspergillus sp.	7.16	35	7.33	36	7.52	38	7.86	40	8.22	44	8.25	49	8.30	48	8.32	38
Penicillium sp.	7.22	33	7.52	34	7.78	36	7.78	38	8.21	48	8.27	52	8.30	52	8.3	52
Aspergillus sp. *+Penicillium* sp.,	7.21	38	7.5	38	7.61	39	7.69	57	7.86	57	8.04	60	8.31	63	8.37	57
Control	7.02	26	7.01	26	7.02	26	7.0	26	7.02	26	7.02	26	7.02	26	7.02	26

Overall mean value

Table 2B: Biodegradative potentials of *aspergillus* sp., *penicillium* 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
Aspergillus sp.	7.11	31	7.41	32	7.61	33	7.83	35	8.01	40	8.04	45	8.07	47	8.11	47
Penicillium sp.	7.12	31	7.39	32	7.53	33	7.55	36	7.7	42	7.86	44	8.13	40	8.15	31
Aspergillus sp. +*Penicillium* sp.,	7.28	37	7.30	38	7.59	40	7.76	45	7.95	55	8.03	57	8.13	57	8.15	40
Control	7.12	22	7.11	22	7.11	22	7.14	22	7.14	22	7.12	22	7.12	22	7.12	22

Overall mean value

Table 3A**:** Biodegradative potentials of *aspergillus* sp., *penicillium* 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
Aspergillus sp.	15	64	13.6	60	8	55	5.5	50	2	58	1.4	57	0.2	55	0.1	55
Penicilliu m sp.	15.82	64	13.8	62	8.5	60	6	58	2.1	56	1.5	55	0.2	55	0.1	52
Aspergillus sp. +*Penicillium* sp.,	20	65	20	60	14	56	8.5	54	2	52	1.74	49	0.8	47	0.1	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 3B: Biodegradative potentials of *aspergillus* sp., *penicillium* 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
Aspergillus sp.	16.8	90	15.4	89	10	86	7	80	2.1	75	1.58	72	0.5	67	0.11	64
Penicilliu m sp.	17.67	88	16.5	85	16.2	78	8.56	72	2.12	66	2.0	57	0.2	51	0.12	48
Aspergillus sp. +*Penicillium* sp.,	20.9	88	19.6	80	18.6	75	12	70	2.5	65	2.1	65	1.1	60	0.14	60
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

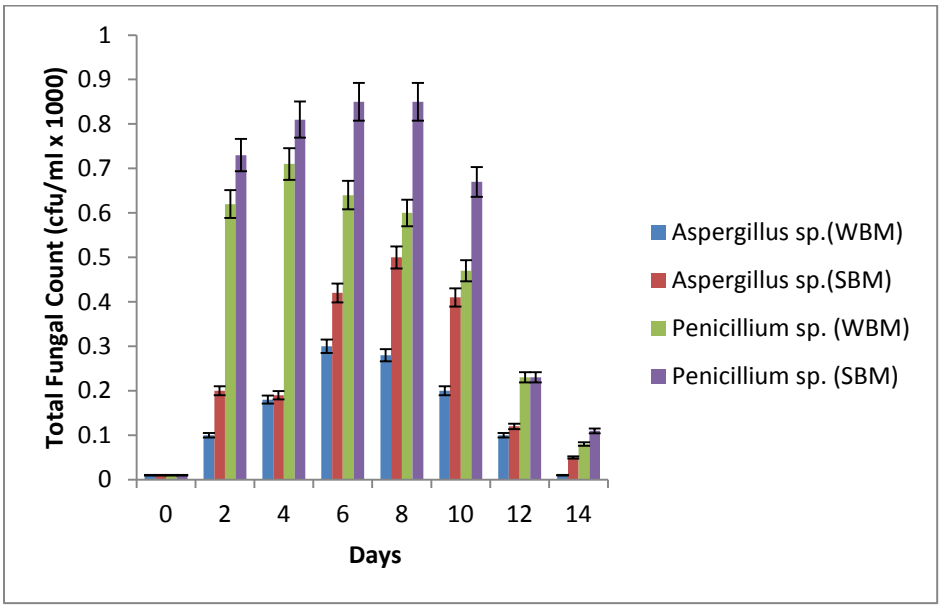

Figure 1: Growth profile of fungal isolates in culture media amended with 2 % synthetic based mud and potassium chloride polymer water based mud.

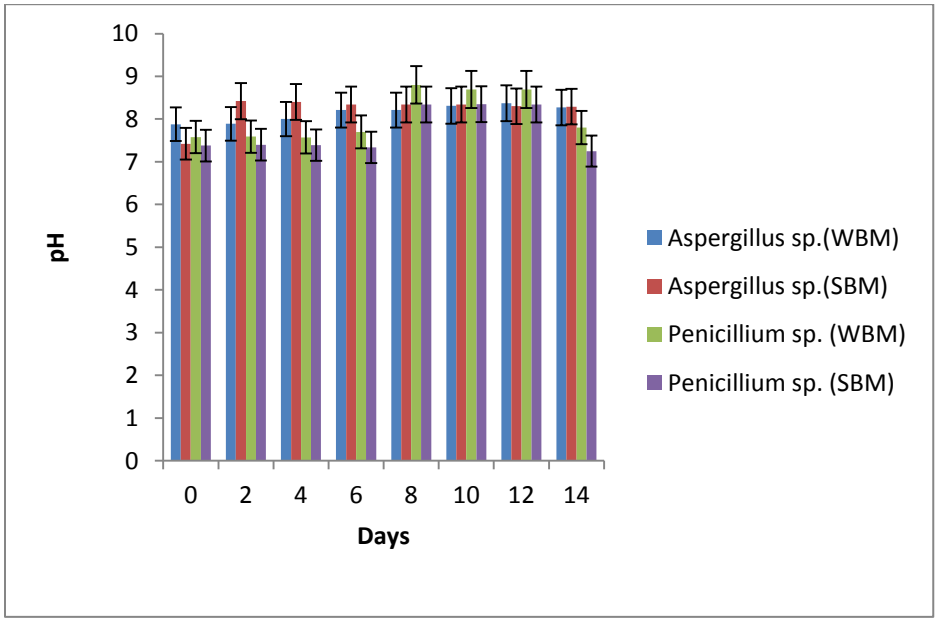

Figure 2: Effect of pH on the growth profile of fungal isolates in culture media amended with 2 % synthetic based mud and potassium chloride polymer water based mud.

The degradation of two different types of drill muds (KCl – polymer water based mud and synthetic based mud) under natural conditions by the indigenous two different fungal genera were examined under natural environmental conditions over a period of 28 days.This investigation has demonstrated that these indigenous fungi harbored by drill cuttings from Ologbo active-wells have the capability to utilize and degrade these drilling muds. Previous investigations have shown that several microorganisms can enhance catabolic activities of hydrocarbon pollutants in the environment [3, 7 and19].

According to [19] 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 *Aspergillus* sp. and *Penicillium* sp. revealed a consistent increase and decrease of the total viable counts (cfu/ml).In this study, the observed high total viable populationscountsof9.1 x 10^2 cfu/ mL at day 16 and 7.9 x 10^2cfu/ mL at day 20 (for broth cultures containing consortium of isolates amended with water based mud; and also with synthetic based mud amendment respectively) are further claims to biodegradation potentials of these isolates. It was also observed that the populations of microorganisms in the experiment were capable of utilizing the drill fluids, as they increased in population at the when the drill muds were introduced but the populations declined gradually upon the degradation of the drill muds. This investigation is line with earlier works [9 and 20] showed that biodegradation of drilling muds increased with higher concentration of fluids. 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 [17]. The observance of high counts in tests containing consortiums indicate that high amount of biodegradation of these muds can be achieve by employing culture containing consortium of isolates rather than single isolates. The pH values of the cultures all 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 [13]. Organic 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 organic carbon in water [22]. The results of the oxidation of the substrates COD (reduced from 88 mg/ l at day 1 to 60 mg/ L at day 28 in synthetic based mud) and BOD_5 (reduced from 20.9 mg/ L at day 1 to 0.14 mg/ L at day 28 in synthetic based mud). Enhanced degradation was observed by fungal consortiums (mixed cultures of fungi)in this study. This may be attributed to the phenomenon of co-metabolic activity of most fungi, where an organism acted as primary utilizers, utilizing substrate molecules while the other acts as secondary utilizers, utilizing the breakdown products of substrate after an initial breakdown by primary utilizers [22]. On a contrary view [16], in a literature report had observed that axenic bacteria were better petroleum degraders compared to mixed bacterial cultures.

The observed growth profiles are again a further evidence of the metabolic potentials of these selected fungal isolates, considering the chemical composition of the mixtures. Increase in total viable counts and pH of the culture media amended with the drilling muds revealed the ability of the existing cells to metabolize the drill muds. However growth of the selected isolates were faster in medium amended with KCl-polymer water based mud than synthetic based mud. This inference is further depicted by the isolates' total viable counts and growth peaks in the experimental culture media.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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