



Effect of Fungi Species on Biodegradability of Developed Drilling Fluids from Baobab Oil and Locally Sourced Bentonite

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Abstract

Drilling mud is an essential element that drives every drilling operation. This study is aimed at evaluation of effect of various fungi species on biodegradability of developed drilling fluids from baobab seed oil and locally sourced Sabon Garin Ngalda bentonitic clay. Mechanical extraction process was employed for the extraction of the oil and then characterized. The drilling fluid was formulated, determined the rheology and filtration properties, then the isolated fungi were introduced to evaluate the biodegradability of the fluids. The characterization results of the oil for specific gravity, sulphur content, kinematic viscosity, pH, flash and fire points were 0.9196, 0.022 ppm, 63 cP, 6.43, 150 °C and 175 °C respectively, which fall within the ASTM D6751-02 standard. The FTIR analysis of the oil was carried out having functional groups of O-H Stretching vibration of alcohol, C-H Stretching vibration of alkanes and C=C Bending vibration of alkenes. The formulated 6g PAC was found to compete favorably with standard API grade having plastic viscosity (PV) of 73 cP, apparent viscosity (AV) of 141.5 cP, yield point (YP) of 137 Ib/100ft² and gel strength (GS) for 10sec and 10min as 6 and 4 Ib/100ft² respectively. The determined filtration properties for Wyoming clay and indigenous sourced Sabon Garin Ngalda bentonitic clay were filtrate volume, cake thickness and mud weight that were found to be 17.6 ml, 2.69 mm and 8.20 lb/gal for Wyoming clay and 6.8 ml, 2.77 mm and 8.25 lb/gal, for 6 g PAC indigenous clay respectively. The biodegradability of the mud samples with respect to the various species of fungi were determined as: 41 %, 55 %, 59 %, 65 %, 78 %, 81 %, 85 %, and 92 % for *Penicillium spp*, *Rhizopus spp*, *Aspergillus niger*, *Fusarium spp*, *Pleurotus spp*, *Trichoderma spp*, *Nigrospora spp* and *Candida albicans* respectively. The findings in this research indicated that formulated drilling mud from baobab seed oil is safe as drilling fluid and has characteristics close to that of the standard drilling mud. It is also biodegradable and can adequately serve as an alternative to the commercial products.

1. Introduction

Hydrocarbons beneath the earth crust are globally recovered from reservoirs through a process of drilling with the aid of complex heterogeneous fluids known as drilling fluids. Drilling fluids play a significant role in aiding the drilling of borehole into the earth [1]. The term drilling fluids or drilling muds generally applies to fluids used to help maintain well control and remove drill cuttings (rock fragments from underground geological formations) from holes drilled in the earth. Drilling fluids are fluids used in petroleum drilling operations. These fluids are a mixture of clays, chemicals, water and oils. These fluids are used in a borehole during drilling operations [2]. The petroleum industry is a key important sector worldwide as a lot of other industries and activities depend on its products and

services. During the extraction of petroleum, an important operation that must be carried out is the drilling of the pathway for laying the pipes through which oil will be produced from the depth of the earth [3]. Depending on the geology of the area, drilling of the ground can be an expensive operation and is often an important factor in the overall cost of operation [4].

The Mineral oil and Diesel oil used as fluid phase of the drilling mud and operational discharges of spent as well as the accumulated drilling cuttings from oil and gas industries are not biodegradable and are considered as source of environmental pollution. Hence there is need for development of biodegradable oil-based drilling fluid from plant sourced oil. Alternative ways of sourcing plant oil are an important issue globally due to efforts sought to reduce over dependent on conventional sourced oil which currently pose strong threat to a clean environment.

The baobab seed oil was selected for this research because it is nontoxic and biodegradable. The Baobab (*Adansonia digitata*) tree has been subjected to intensive scientific studies considering its agricultural, industrial and medical importance. The seed has been reported to be eaten raw or roasted [5], having a nutty flavor and this can be a substitute for Coffee and Ground nut seeds. The seed kernels contain golden yellow oil that can be obtained through screw pressing method [6].

Bioremediation is the use of naturally occurring microorganisms or genetically engineered microorganisms (bacteria and fungi) by man, to detoxify man-made pollutants [7]. There has been increasing interest by researchers in the application of organisms and nutrients to contaminated soils for effective biodegradation of oil. Naturally occurring microbial communities that respond to the presence of contaminating hydrocarbons normally have more than one type of hydrocarbon utilizing microorganisms [8, 9]. Fungi infection of oily seeds has been reported to deteriorate oils extracted from seeds [10]. Properties such as the free fatty acid content peroxide and saponification values, as well as, oil quantity are known to be reduced by microbial infections [11]. Such microorganisms use the oil as sources of carbon and energy for metabolism [10]. The effect of oil on microbial population depends upon the chemical composition of the oil, the nature of the species and sometimes the population of microorganisms. The degradative end product of any oil is distinct depending on the degradation pathways. The ability of *Aspergillus* species on crude oil, diesel, spent engine oil, kerosene and many oil extract have been reported [12].

This study is aimed at evaluation of effect of various fungi species on biodegradability of developed drilling fluids from baobab seed oil and locally sourced Sabon Garin Ngalda bentonitic clay. This can be achieved through determination of the rheological and filtration properties of the formulated drilling mud, determination of its bio-degradability and investigation of different type of fungal species on biodegradability of the formulated drilling mud.

2. Materials and Methods

The major materials used in this research are; Baobab oil, Sabon Garin Ngalda bentonite (obtained from Yobe state, Nigeria) and Wyoming grade bentonitic clay, poly anionic cellulose (PAC), Barium Sulphate (Barite), Gum Arabic and distilled water.

2.1 Oil Extraction and Characterization

2.1.1 Sample preparation

Baobab fruit was purchased from market at Zaria, Kaduna, Nigeria. The pulps were manually separated from the seeds using a knife and dissolved in water, then dried under the sun. The seeds were ground to smaller size to pass through a 40-mesh screen, then placed in an airtight plastic container and stored at ambient temperature for further processing.

The technique for oil extraction by mechanical presses was used. In this method, an engine driven screw press was used. The mechanical oil extraction technique was conducted using a mini 40 screw press at centre as depicted in Figure 1.

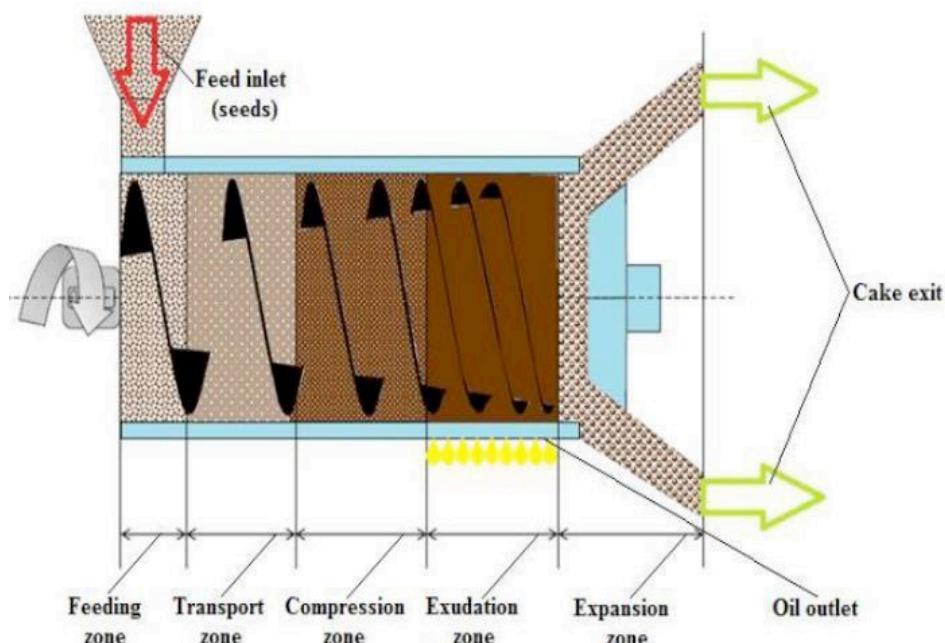


Figure 1: Screw press Mechanical Extraction Process [13].

2.1.2 Determination of specific gravity

An empty specific gravity bottle was initially weighed, followed by addition of baobab oil into the bottle. The oil was then substituted with water of the same volume and reweighed. The specific gravity was determined by calculation using equation 1.

$$\text{Specific gravity} = \frac{M_o - M_c}{M_w - M_c} \quad \text{Eqn 1}$$

Where:

M_o is the mass of baobab oil and the bottle,
 M_w is the mass of water and bottle and
 M_c is the mass of empty specific gravity bottle.

2.1.2 Determination of sulfur content

The oil was poured into the sample cell, placed the sample cell on the sample holder, set to the measurement condition, pressed the "MEAS" button to start the measurement, and instrument displayed the results [14].

2.1.3 Determination of kinematic viscosity

The sample was filtered through a sintered glass (five meshes to eliminate solid materials in the liquid oil). This viscometer was charged with the oil by inverting the tube's thinner arm into the oil and suction force was drawn up to the timing mark of viscometer. Afterwards, the instrument was turned to normal vertical position. The viscometer was placed into a holder and inserted to constant temperature both set at 40°C and allowed for 5 minutes for temperature to attain same value (40 °C). Suction pressure was applied to the thinner arm to draw the liquid little above the upper timing mark.

Readings of afflux time of the flow of the oil as it flows freely from the upper timing mark to the lower timing mark were recorded.

2.1.4 Determination of flash and fire point

The sample oil was poured into the cup to the filling mark, the cup was placed on the tester and closed. The thermometer and stirrer were placed in their positions on the tester and test was started. Heat was applied to the sample in the test cup at the range of 5 °C to 6 °C per minute with help of heat regulator, the sample was heated gradually at the same time stirring the specimen at the rate of rotation 60 per minutes. Look carefully on the thermometer when the temperature reaches 17 °C below the expected flashpoint, the test flame was applied. The bead of the test flame was 4mm and stirring was stopped while applying the test flame. The test flame was then applied at 1°C, a unique flash will appear while applying the test flame with respect to the gradual increase of temperature, the temperature at this point reading was taken as the flash point of the sample. Now heat was continue applied and the test flame at temperature rising for every 2°C. Note the temperature reading on the thermometer as fire point of the sample when the sample catches fire and burns at least for 5seconds. The analysis was repeated 3-times, the average values were taken as the flash and fire points of the sample [15].

2.2 Drilling fluid formulation

The drilling fluid formulation of [16] using 60:40 ratio of baobab oil and distilled water respectively, as the based fluids as depicted in Table 1. The measured materials for each formulation were thoroughly mixed using Hamilton Beach Mixer until homogenous mixtures were achieved. The formulated fluids were then stored into mud sample containers, labelled as 1, 2,3,4,5,6,7 and 8 and allowed to age for 24 hours. These formulations are for the eight different fungi species.

Table 1: Composition of Drilling Muds

Drilling Fluid Constituents	Measured Quantity
Baobab oil (ml)	210
Distilled Water (ml)	140
Bentonite (g)	24.5
Gum Arabic (g)	2.0
Polyanionic cellulose (PAC) (g)	2.0
Barium sulphate (g)	10

By testing the properties of each run, the properties were determined and compared with formulations from API Wyoming bentonite.

2.2.1 Viscosity, Gel strength and Yield point Measurement

Viscosity and gel strength were measured using low temperature viscometer (FANN 35SA) which is a rotational instrument powered by an electric motor, it allows speeds of 3, 6,100,200,300 and 600 rpm, the reading from rotor sleeve speeds of 300 and 600 rpm was used to determine plastic viscosity and yield point, and 3 rpm, 6 rpm was used to determine the gel strength [17].

1. Apparent Viscosity (in centipoise-cP):

$$\text{Apparent Viscosity (AV)} = \frac{\text{Reading @ 600 rpm}}{2} \quad \text{Eqn 2}$$

2. Plastic viscosity (in centipoise-cp):

$$\text{Plastic viscosity (PV)} = \text{Reading @ 600rpm} - \text{Reading @ 300rpm} \quad \text{Eqn 3}$$

The yield point was determined computationally taking the difference between dial readings at 300 rpm and plastic viscosity.

3. Yield point (in lb/100ft²):

$$\text{Yield Point (YP)} = \text{Reading @ 300} - \text{Plastic Viscosity} \quad \text{Eqn 4}$$

The gel strength was determined using FANN 35SA viscometer. 10 seconds and 10-minute gel strength were measured. The formulated sample was poured into the sample holder and mounted to position and the base lifted until the mud reach the scribe line and the lock screw tightened. The sample was subjected to shear at 600 rpm for 10 seconds and the gear was then set to neutral position. The motor was shut off and waited for 10 seconds and the deflection at 3 rpm is recorded as 10 seconds gel strength in lb/100 ft². This procedure was repeated for the 10-minute gel strength.

2.2.2 Determination of Fluid Loss

Low temperature API filter press was used for fluid loss measurement. The sample drilling fluid was poured into the API filter press cell to about three quarter of the cup. The filter cell was then placed in the frame. The top cap was placed to ensure that the gasket was firmly secured in place. The top cap was then placed to ensure that the casket was firmly secured in place and graduated cylinder placed under the drain tube to collect the filtrate. Having ensured that the unit was tight a pressure of 100 psi supplied by mini-CO₂ cartridge was applied to the filter cell through a regulator and the filtrate collected after 30 minutes. The volume of filtrate was read from the graduated cylinder in cm³. The system was then relieved of the pressure and the cell removed, mud is then discarded and the filter paper replaced for another round [17].

2.2.3 Fungi Isolation, Identification and Determination of Biodegradability

The fungi were sourced from various sources as indicated in **Table 2**. The substances were collected into a tin and then transported to the laboratory. Sabouroud Dextrose Agar (SDA) was prepared by weighing 20g of the SDA powder using a digital weighing scale. The powder was dissolved into 500ml of distilled water (base on the manufacturers instruction). The solution was autoclaved at 121 °C and 15psi for 15 minutes. The pressured steam was let out through the autoclave valve to allow cooling of the internal compartment and for precautionary measures. The autoclaved SDA solution was brought out of the autoclave and poured into a petri dish (at an estimated volume of 20ml each). It was allowed to cool and solidify for 1-hour. This is to achieve the solid state of the agar. The media were placed in a hot air oven, for the purpose of drying and removal of moisture from the surface of the agar at 50°C. The fungi (from various sources) were inoculated on the prepared Sabouroud Dextrose Agar (DAD), by using a wire loop (forceps). It was incubated at 37 °C for 7-days. The Inoculated media was brought out of the incubator after 7-days [18]

The smeared of each Isolate was made on a glass slide using a drop of saline and the isolate. The slides were stained with lacto phenol cotton blue for 20min (standard operation procedure). The slides were washed under running tab water and allowed to air dry. Oil immersion was added to the stained smear and viewed under the microscope using the x100 objective lens of the microscope. The

fungi were then identified using their hyphae formation and presentation [18]. The various sources and their respective fungi used are presented in **Table 2**.

Table 2: Fungi and their sources

Sources	Fungi
1. Decayed banana	<i>Fusarium</i> spp
2. Decayed apple	<i>Penicillium</i> spp
3. Decayed bread	<i>Rhizopus</i> spp
4. Decayed vegetables	<i>Pleurotus</i> spp
5. Decayed wheat	<i>Trichoderma</i> spp
6. Human sample	<i>Candida albicans</i>
7. Mould	<i>Aspergillus niger</i>
8. Aerobic spore bearer	<i>Nigrospore</i> spp

The biodegradability of the sample drilling mud containing different species of fungi were determined by taking ratio of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) multiplied by 100 as shown in equation 5.

$$\text{Biodegradability} = \frac{\text{BOD}}{\text{COD}} \times 100. \quad \text{Eqn 5}$$

3. Results and Discussion

3.1. Baobab Oil Percentage Recovery

The baobab seed was found to contain an appreciable 21% of oil content, hence, considered as one of the major additional sources of oil. This result is close to the results reported by several authors which falls within the range of 22.5 – 23% [19, 20]. In general, the oil content of baobab kernel varies due to difference in species and environmental factors. On the other hand, [21] has reported an oil content of 12.2% which is significantly less than result reported herein. In addition, higher oil content of 25.7% and 32% have been reported [22, 23].

3.2 Physicochemical Properties of Baobab Oil

The physical and chemical properties of baobab seed oil extracted are depicted in **Table 3**. The kinematic viscosity of baobab seed oil at 40°C was found to be 63cSt contrary to the findings of [24] that reported 33.69cSt. The specific gravity of extracted baobab seed oil was found to be 0.9196, which is slightly below what [25] and [20] reported as 0.937 and 0.928 respectively. The sulfur content was found to be 0.022 ppm, which is in trace and negligible. The flash point is the minimum temperature at which a volatile material can vaporize to form an ignitable mixture in air. The extracted Baobab oil has flash point of 150°C, which falls within the standard range as depicted in **Table 3**. The fire point of baobab seed oil was found to be 175°C. Both flash and fire points are factors to be considered for the purpose of safety and transportation. The pH of 6.48 was recorded, which means it is slightly acidic and also less acidic compared to what [24] reported as 5.6. This difference might occur due to species and environmental conditions

3.2.1 Fourier Transform Infrared (FTIR) Result

The result of the Fourier transform – infrared (FT-IR) of the Baobab seeds oil is presented in **Figure 2**, having frequencies of 3734.8cm⁻¹ indicating O-H Stretching alcohol, 3008.0cm⁻¹ is C-H

Stretching alkane, 2922.2cm^{-1} for C-H Stretching alkane, 2855.1cm^{-1} which is C-H Stretching alkane, $2009.\text{cm}^{-1}$ for N=C=S Stretching Isothiocyanate and 1941.9cm^{-1} for C=C=C Stretching alkene. The result indicates that the functional groups contain in the baobab oil are alcohol, alkane, alkene, Isothiocyanate, Cyclopentanone, Sulfonete, Amine Aliphatic ether and Carboxylic.

Table 3: Physicochemical Properties of Baobab Oil

S/No	Analysis	Value	ASTM D6751-02
1.	Specific Gravity	0.9196	0.968
2.	Sulfur (ppm)	0.022	
3.	Kinematic Viscosity(cP)	63	
4.	pH	6.48	
5.	Flash Point($^{\circ}\text{C}$)	150	>130
6.	Fire Point ($^{\circ}\text{C}$)	175	
7.	Color	light yellow	
8.	Od or	Pleasant odour	

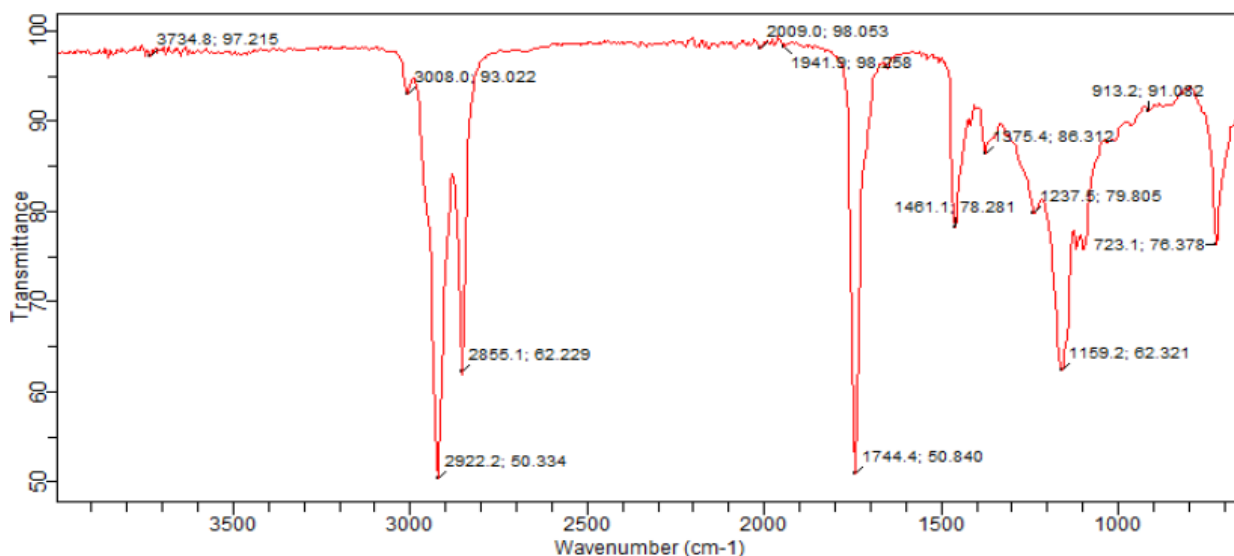


Figure 2: FTIR Analysis of Baobab seed oil

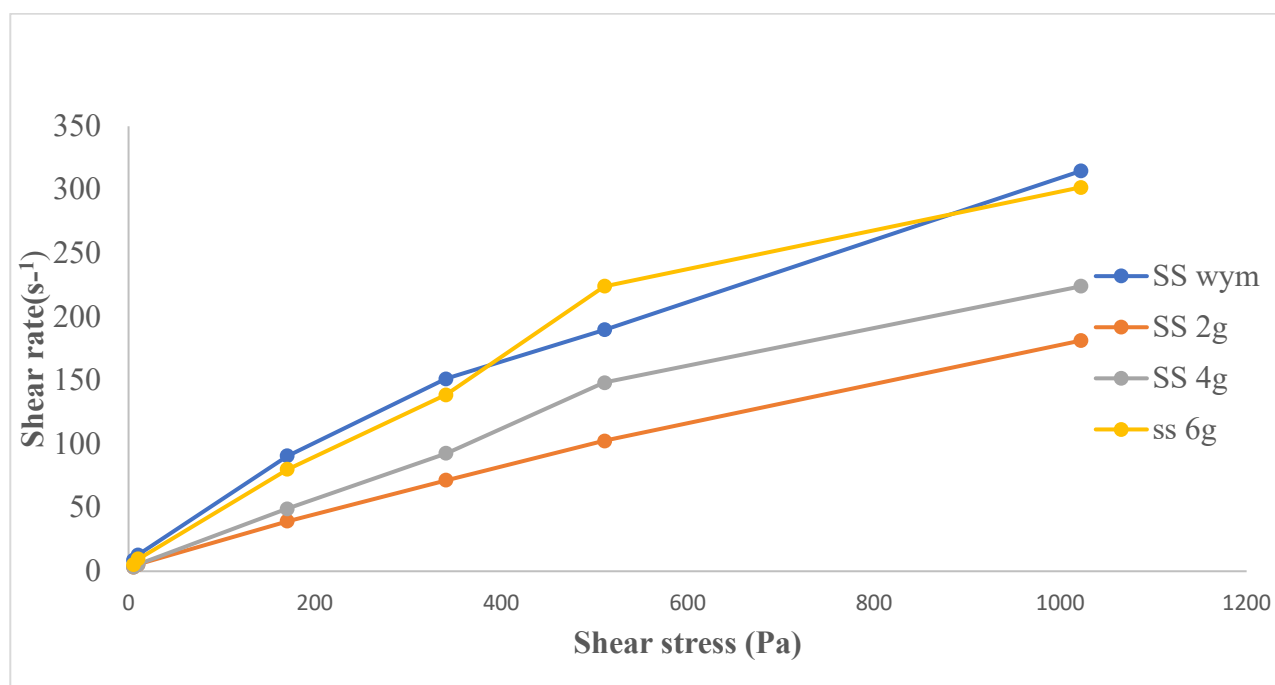
3.3 Rheological Properties of Formulated Drilling Mud

The rheological properties (plastic viscosity (PV), apparent viscosity (AV), yield point (YP) and Gel Strength) measured were at 2g, 4g and 6g PAC and compared with the standard (API grade formulation) as depicted in Table 4. It was observed that at 6g PAC, the PV, AV, YP and GS were found to be 73cP, 141.5cP, 137lb/100 ft² and 6/4lb/100 ft² respectively. The AV for 6g PAC formulation was to the standard grade, with the exception of PV, YP and GS. The shear stress shear rate data was also computed as shown in Figure 3. From the consistency curves, it was observed that at 6g PAC, the trend coincides with that of the Wyoming consistency trend, while that of 2g and 4g PAC are below that of the Wyoming consistency trend.

Table 4: Rheological Properties of Formulated Drilling Mud

Parameter	Drilling Mud 2g-PAC	Drilling Mud 4g-PAC	Drilling Mud 6g-PAC	Wyoming (Standard)
PV (cP)	74	71	73	117
AV (cP)	85	105	141.5	142.5
YP (lb/100 ft ²)	22	62	137	61
GS(lb/100ft ²)	10sec 2	3	6	8
	10min 4	4	4	12

Key: PV- Plastic Viscosity, AV- Apparent Viscosity, YP- Yield point and Gel Strength.

**Figure 3:** Variation of Shear rate with Shear Stress for 60:40 formulation

The filtrate volume recorded for both Wyoming Clay formulation and 6g PAC Sabon Garin Ngalda (locally sourced) clay are depicted in Figure 4. Plotting the filtrate volume versus time allows for the filtration period of 30 minutes.

A total filtrate volume for locally sourced bentonite formulation and the standard Wyoming Clay formulation were 6.8ml and 17.6ml respectively. The result indicates that, the locally sourced clay has better filtration properties than the Wyoming Clay by losing less fluid. [26] used an analytical approach and parameters such as fluid rheological properties, fracture parameters, and operational conditions to quantify fluid losses in induced fractures. Their sensitivity analysis revealed that an induced fracture width and rate of fracture propagation depends on the net differential pressure across the fracture and stiffness of the fracture. The filter cake around the wall of the well and inside the fractures has properties that enable them to act as a stable plug to impede subsequent mud and filtrate

loss. Those properties include thickness, cohesion, and tightness. Filter cakes are elastic in nature and can undergo plastic deformation under a shear failure condition. The cohesive property of a filter cake is sometimes referred to as compressive strength, and the primary source of filter cake's cohesive strength is particle to particle contact which [27] and [28] referred to as particle-particle and particle-pore interlocking. However, the thickness of the cake for local sourced clay was found to be 2.77mm, while that of the Wyoming clay was found to be 2.69mm.

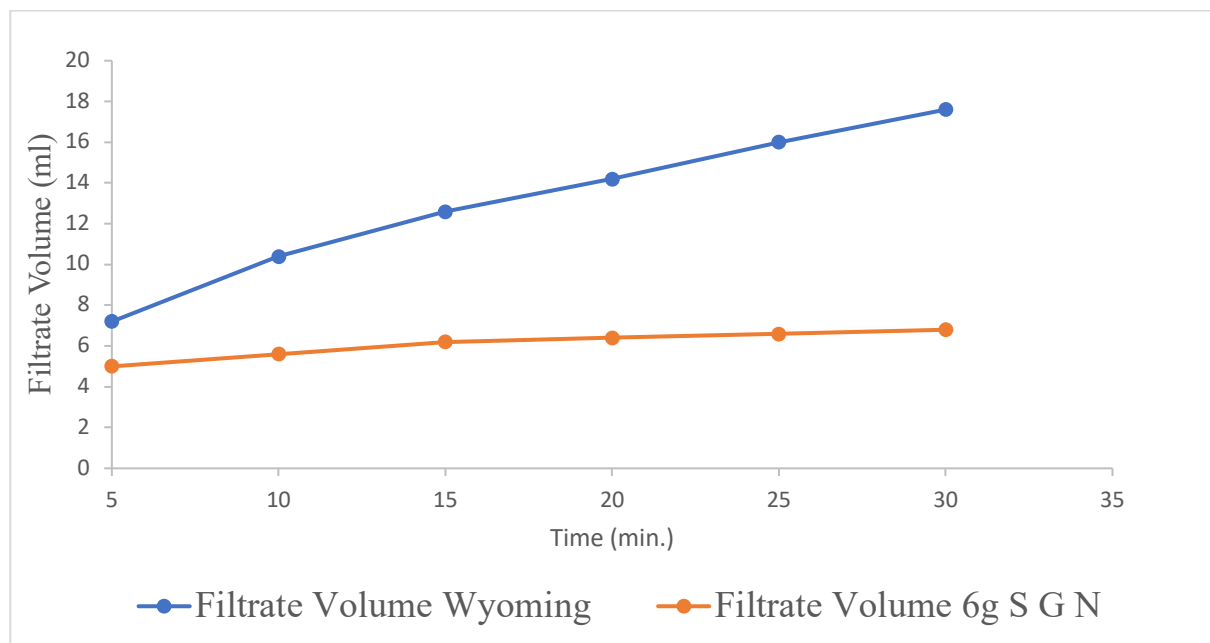


Figure 4: Effect of Filtrate Volume with Time for Wyoming Clay and 6g Sabon Garin Ngalda Bentonitic Clay

The mud density for both Wyoming and locally sourced clay were found to be 8.20lb/gal and 8.25lb/gal respectively. The mud weight could reach up to 22 or 23lb/gal depending on the formation pressure [29].

3.3 Biodegradability of Formulated Drilling Mud

Eight different species of fungi were inoculated into drilling mud and allowed to grow for the incubation period of thirty days. After each five days viscosity readings of the mud were noted and recorded. When fungi grow as free mycelia, a significant increase of apparent viscosity is observed [30]. This increase may change the rheological behavior; transforming the culture media to a non-Newtonian fluid. This fact induces an important reduction on mixing and air dispersion, which causes limitations on solid-fluid mass transference and oxygen availability within the liquid medium. The sample fungi population showed similar trend pattern of growth with a slight inconsistency around 25 to 30days with respect to *Aspergillus Niger*, *Rhizopus* spp., *Pleurotus* spp., and *Candida albicans*, this is due to increase in apparent viscosity as depicted in Figure 5. The pH of the formulated drilling fluids containing the fungi species; *Aspergillus Niger*, *Fusarium* spp., *Penicilium* spp., *Rhizopus* spp., *Pleurotus* spp., *Trichoderma* spp., *Nigrospora* spp., and *Candida albicans* were (6.34, 6.12, 6.25, 6.06, 6.06, 6.30, 6.39, and 6.18;) respectively.

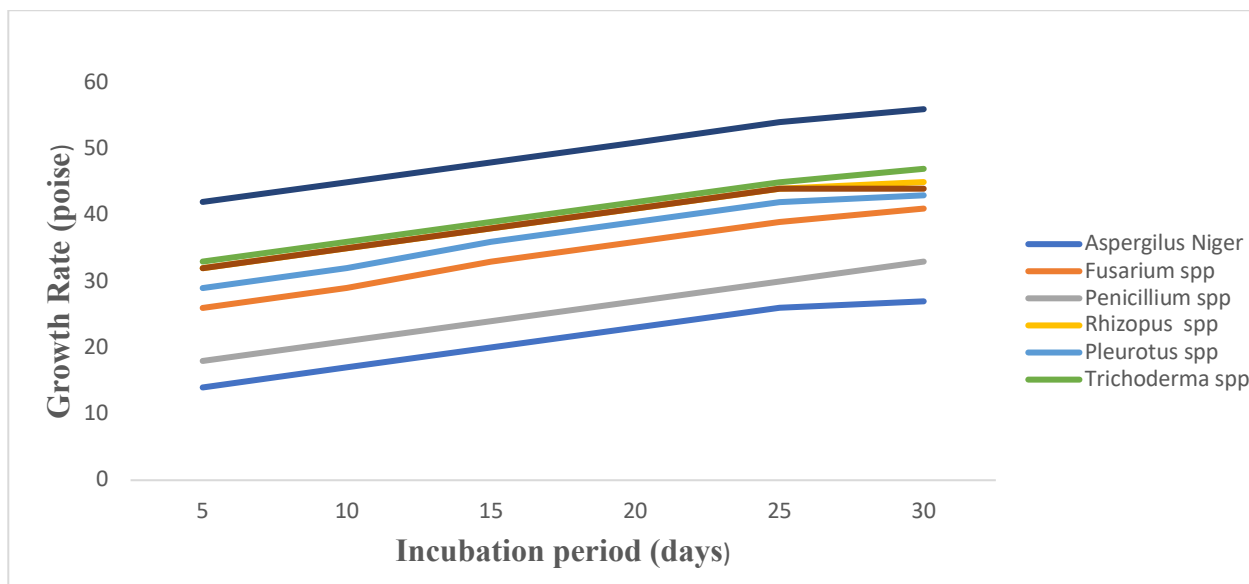


Figure 5: Rate of growth and incubation period of Fungi Species

Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined and recorded for each of the fungi species. The biochemical oxygen demand is the amount of oxygen consumed by bacteria and other micro-organism while decomposing organic matter under aerobic condition at a specified temperature, while Chemical Oxygen Demand is amount of oxygen require to decompose organic matter in waste water. Findings in this research have shown that formulated drilling fluid from baobab seed oil is safe as drilling fluid and has characteristics close to that of the standard drilling mud. Figure 6 depicts the BOD / COD of the various species of fungi used. The blue color bars stand for the BOD while the maroon color bars stand for the COD respectively.

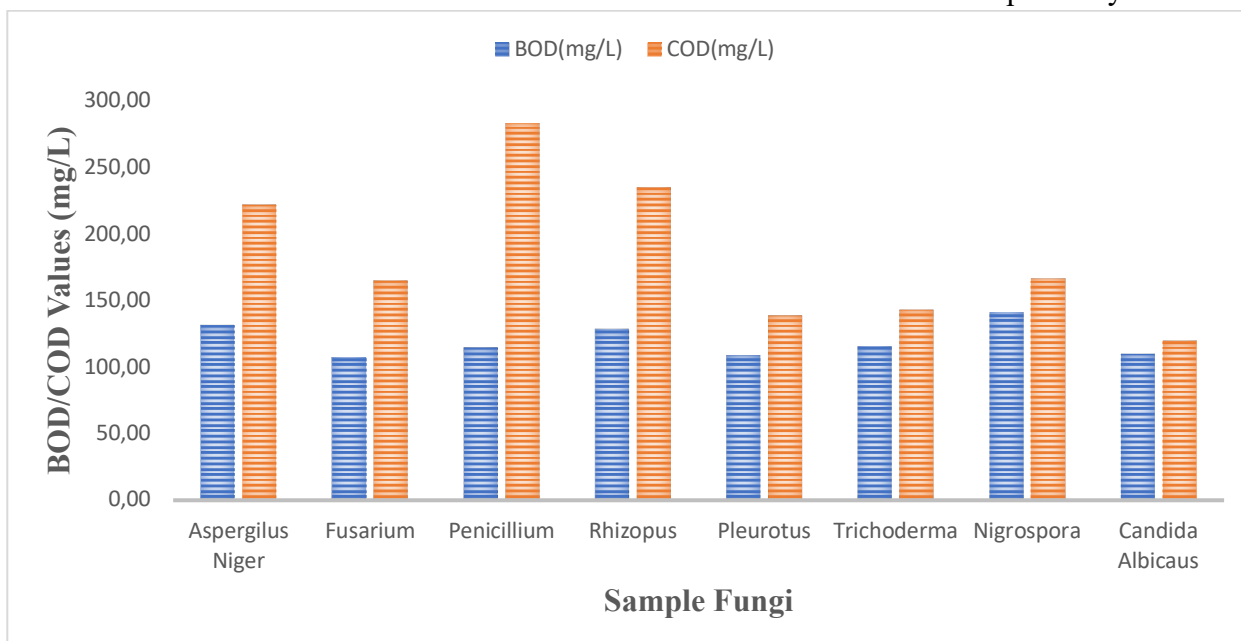


Figure 6: BOD/COD Values of formulated drilling mud for different species of Fungi

The biodegradability of the sample drilling mud containing different species of fungi were determined using equation 5.

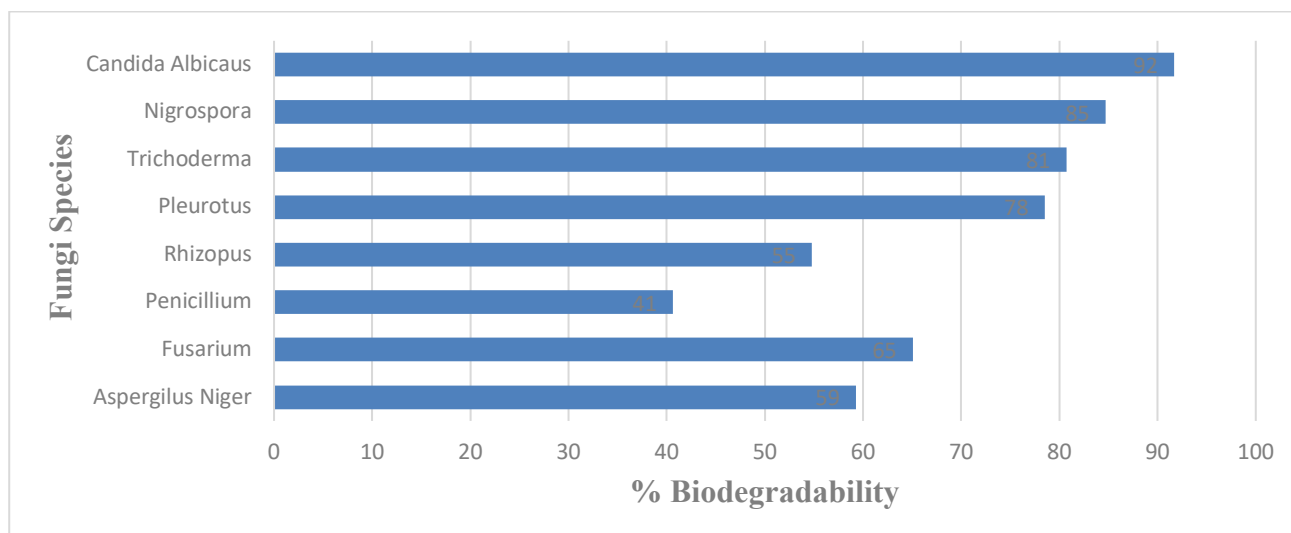


Figure 7: Effect of Biodegradability of different fungi species

Figure 7 depicts the effect of biodegradability of eight different fungi species in the sample drilling mud. The most effective species is *Candida albicans* which has 92% biodegradability, followed by *Nigrospora* 85%, *Trichoderma* 81%, *Pleurotus* 78%, *Fusarium* 65%, *Aspergillus Niger* 59%, *Fusarium* specie 65% and *Penicillium* specie having the least biodegradability of 41% respectively.

Conclusion

Oil extracted from Baobab seeds using Screw press Mechanical extraction process yielded a reasonable quantity of 21%. The Characterized baobab seed oil revealed that the specific gravity, sulphur content, viscosity, pH, flash and fire points were; 0.9196, 0.022ppm, 63cSt, 6.43, 150°C and 175°C respectively. The FTIR results revealed that the dominant compound in all the samples were alcohol, alkane, alkene, protein, cyclopentanone, and aliphatic amines functional groups. The rheological properties of the formulated drilling mud (6g PAC) were found to be 73cP, 141.5cP, 137 lb/100ft² and 6/4 lb/100ft² for PV, AV, YP and GS respectively. Mud weight, filtrate volume and cake thickness were 8.25lb/gal, 6.8ml and 2.25mm respectively. The biodegradability of the mud with respect to the various species were 41%, 55%, 59%, 65 %, 78 %, 81 %, 85 %, and 92 % for *Penicillium*, *Rhizopus*, *Aspergillus Niger*, *Fusarium*, *Pleurotus*, *Trichoderma*, *Nigrospora* and *Candida albicans* respectively. The effectiveness of the biodegradability of the mud depends on the type of fungus specie. The most effective species were *Candida albicans*, *Nigrospora*, *Trichoderma* and *Pleurotus* having 92 %, 85%, 81 % and 78 % biodegradability.

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