IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 2 Ver. I (February. 2018), PP 72-82
www.iosrjournals.org

Cumulative percentage Ultimate biodegradability of drilling fluids in brackish and marine ecosystem

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***Abstract**: Cumulative percentage Ultimate biodegradability of two types of Drilling fluid – water base drilling fluid (WBDF) and oil base drilling fluid (OBDF) used in the upstream sector of the Nigeria petroleum industry were investigated in brackish and marine ecosystems. The rate of ultimate biodegradability was estimated from the ratio of Biochemical Oxygen Demand to Chemical Oxygen Demand. Evaluation of cumulative percentage (∑%) ultimate biodegradation of the drilling fluid in brackish water were; WBDF (25.74), OBDF (12.82), while in marine water, WBDF (5.46), OBDF (1.84). Analysis of comparative percentage ultimate biodegradation rate revealed that WBDF is 2.01 times more biodegradable than OBDF in brackish water environment [WBDF 66.75%, OBDF 33.25%] whereas in marine ecosystem WBDF is 2.97 times more biodegradable than OBDF [WBDF 74.79%, OBDF 25.21%]. It further shows WBDF having higher differential rate of biodegradability in marine than brackish (that is, WBDF is more readily biodegradable than OBDF in marine water than brackish water).Drilling fluid utilizing bacteria genera isolated were; Pseudomonas, Bacillus, Micrococcus, Enterobacter and fungi; Aspergillus, Penicillium, Rhizopus and Mucor. Owing to these findings, water base drilling fluid is the best option in terms of eco-friendliness both in brackish and marine ecosystem.*

***Keywords**: Cumulative Percentage (∑%) Ultimate biodegradability, water base drilling fluid, oil base drilling fluid, Drilling fluid Utilizing bacteria and fungi.*

Date of Submission: 09-02-2018 Date of acceptance: 26-02-2018

I. Introduction

The petroleum industry is a complex combination of interdependent operations including exploration and production operations, the processing of crude oil into consumers products, transportation and marketing activities. Oil and gas (petroleum) industry comprises of two main parts (sector): 'Upstream' – the exploration and production (E&P) sector of the industry; and 'Downstream' – the sector which deals with refining and processing of crude oil and gas products, their distribution and marketing (UNEP IE, 2006). Drilling fluids are mixture of natural and synthetic chemical compounds used to cool and lubricate the drill bit, clean the hole bottom, carry cuttings to the surface, control formation pressure and improve the function of the drill string and tools in the hole (Burke, 1995). In Nigeria, two types of drilling muds – water based, and oil based (mineral/synthetic or pseudo) muds are used, (EGASPIN 2002). Off-shore drilling is now common to explore new oil resources and this constitutes another source of petroleum pollution (Readman *et al.,* 1992). Environmental pollution with petroleum and petrochemical products has been recognized as a significant and serious problem (Alexander, 2002). Most components of oil are toxic to humans and wildlife in general, as it is easy to incorporate into the food chain. This fact has increased scientific interest in examining the distribution, fate and of oil and its derivatives in the environment (Alexander, 2002; Semple *et al.*, 2003; Stroud *et al*., 2007, 2009; Nrior and Odokuma, 2015a). Petroleum products employed in upstream sector of the Nigeria petroleum industry include drilling fluid (oil base and water base), oil spill dispersants, degreasers and industrial detergents (EGASPIN 2002). Biodegradation is the breakdown of an organic substance such as oil, by the action of living organisms usually micro-organisms and especially bacteria (Cornelissen and Sijm, 1996) some substances biodegrade more rapidly and more completely than others. Biodegradation may occur under both aerobic (with oxygen) and anaerobic (without oxygen) conditions. Biodegradation is an important factor for reduction and removal of organic contaminants from the environment. The evaluation of biodegradability of anthropogenic organic substances is an essential parameter for environmental risk assessment and required according to appropriate legislation [European Parliament Regulation (EC), 2004]. The natural environment is predominantly aerobic, which for a long time has led to a focus on the biodegradation behavior of chemicals under aerobic conditions. Thus, a number of international recognized standard test methods regarding the aerobic biodegradability of substances have been developed.

Aerobic Hydrocarbon Biodegradation has been commonly accepted as the major mechanisms of removal of hydrocarbons from many contaminated environments. Aerobic organism like *Pseudomonas*, *Flavobacterium*, *Moraxella*, *Marinobacter* and *Vibrio* (Gautheir *et al*., 1992) were found to degrade PAH (Polynuclear Aromatic Hydrocarbons) under aerobic marine conditions. *Pseudomonas testosterone, Psendomonas putida and Psendomonas stutzeri biovars* are capable of degrading naphthalene (Garcia Valdes *et al*. 1988). All these organisms listed are all classed as eubacteria. Anaerobic Hydrocarbon Biodegradation uses sulphate reducing bacteria. Also, *Pseudomonas*, *Proteus, Micrococcus*, *Bacillus*, *Rhizopus*, *Aspergillus* and *Penicilium* were capable of degrading Oil spill dispersant and drilling fluids (Nrior and Wosa, 2016; Nrior, *et al.*, 2017a, b)

A changing pattern of anthropogenic activities is linked to inconsistencies in the relationship of ecological systems in the environment. The choice of fossil fuel materials used in energy production is directly responsible for increases in carbon dioxide and other gases resulting in the current trend of global warming (Nrior *et al*., 2017a). The evaluation of biodegradability of anthropogenic organic substances is an essential parameter for environmental risk assessment and required according to appropriate legislation [European Parliament Regulation (EC), 2004]. New petroleum products employed in the upstream sector of the Nigeria Petroleum Industry whose biodegradability are low and ecotoxicological indices are high constitute great harmful effects in the tri-aquatic environment (Nrior et al., 2017b)

The aim of this study is to evaluate and compare the biodegradability potentials of drilling fluids – water base and oil base and the possible discharge dilution in marine, brackish and fresh water environment.

II. Materials and Methods

Source of water samples

Brackish and marine water samples were collected separately with sterile plastic ten (10) litre containers. Each sample bottle was rinsed with the appropriate sample before the final collection according to the standard methods. To collect the water sample, the base of the sterilized sample bottle was held with one hand, plunged about 30 cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow [APHA, 1998].

Brackish water samples were collected from Trans-Amadi Industrial Layout River near Port Harcourt zoo in Port Harcourt Local Government Area in Rivers State, Nigeria. The river spans from Rumuogba via Woji, Azuabie down to Marine Base, Port Harcourt. The river does not only receive faecal (as the coastal dwellers traditionally defecate into the water body), other industrial chemicals, solids and waste water of domestic origin, but also serves as the sink for used drilling fluid, degreasers, dispersants and Industrial detergent waste water. Marine water samples were collected from Bonny Sea in Bonny Local Government Area in Rivers State, Nigeria. The containers were rinsed three times with the water samples to be collected at the site before collection was made. The sea spans from Okrika Main River to Bonny Island and empties into the Atlantic Ocean. The sea does not only receive fecal (as the coastal dwellers traditionally defecate into the water body), other industrial chemicals, solids and waste water of domestic origin, but also serves as the sink for used petroleum products and all kinds of effluents.

Source and types of drilling fluids

The drilling fluid, water based and oil based drilling fluids were collected with sterile plastic containers from Adax petroleum company, Izombe well Owerri, Imo State Nigeria.

Source of microorganism for biodegradability test

The microorganisms for the biodegradation study were naturally occurring micro flora in the specified aquatic environment.

Biodegradation test procedure

This was carried out in accordance with standard methods used by Nrior and Odokuma (2015b). Three hundred milliliter (300ml) each of brackish and marine water sample collected from Azuabie River and Bonny sea respectively were dispensed into three 500ml Erlenmeyer flask for each water type given a total of six (6) flask for biodegradability set-up. After that, for each set of three [for each water type]; 1% (3ml) each of the Water base drilling fluid and oil base drilling fluid were dispensed into the two (2) separate flask. The third (3^{th}) flask was not contaminated with any test toxicant and was used as a control. The flasks were perforatedly plugged to allow for aeration, and were kept at ambient temperature (28 ± 2^0C) for 28 days.

Samples were taken at day 0, 5, 10, 15, and 20 from the experimental test flasks to determine the pH, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Heterotrophic Bacterial Counts, Total Fungal Counts, and Drilling Fluid Utilizing Bacteria.

Microbiological analysis

Isolation and enumeration of total heterotrophic bacteria

Total heterotrophic bacteria for each biodegradation set up were enumerated by spread plate method. A 0.1ml aliquot of the 10^{-1} to 10^{-4} was transferred unto well-dried nutrient agar plates and incubated at 37^0C for 24 to 48 h. after incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates using the streak plate technique. Discrete colonies on the plates were aseptically transferred into agar slants, properly labeled and stored as stock cultures for preservation and identification (Odokuma and Ibor, 2002; Nrior and Wosa, 2016).

Isolation and enumeration of total fungal count

The total fungi population in the biodegradation set up (Habitat water sample and oil spill dispersant) were enumerated and isolated by inoculating 0.1ml aliquot of the mixture unto well-dried potato dextrose agar containing antibiotics (Tetracycline, Penicillin and Ampicillin) to inhibit bacterial growth. Pure cultures of the fungi iso- lates were enumerated and transferred unto potato dextrose agar slants as stock cultures for preservation and identification (Odokuma and Okpokwasili, 1992; Nrior and Wosa, 2016).

Isolation and enumeration of Drilling Fluid utilizing bacteria

Enumeration of Drilling Fluid utilizing bacteria was performed by inoculating 0.1ml aliquot of the dilutions unto minimal salt agar plates containing the drilling fluids (Odokuma and Okpokwasili, 1992; Nrior and Wosa, 2016; Nrior *et al*., 2017). Colonies were counted after 48 to 72 h incubation at ambient temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plate.

Identification of bacterial and fungal isolates

The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology (1994). The morphological and biochemical test include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and Voges proskauer tests. The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates of the biodegradation flask set up (Cheesbrough, 2006).

Ultimate biodegradation monitoring using the percentage ratio of BOD to COD

The biochemical oxygen demand (BOD) of each biodegradation test set up was monitored (APHA, 1998) at 0, 5, 10, 15, 20 days. The chemical oxygen demand was determined at day 0. The ultimate biodegradability (Swisher, 1987; Nrior and Odokuma, 2015b) also referred to as the percentage of carbon in the material that is potentially mineralizable was calculated from the percentage of the ratio of BOD (for day 0, 5, 10, 15, 20) to COD at day 0. The percentage of mineralizable carbon in the test compounds that was actually mineralized was derived from this formula,

$$\frac{P \times 100}{I}$$

100 – M = N

P = percentage of potentially mineralizable carbon in the test compound

I = percentage of potentially mineralizable carbon in the test compound at day 0

N = percentage of potentially mineralizable carbon in test compound that was actual- ly mineralized.

III. Result

The value of some physical and chemical properties of the three aquatic environments used for the study was presented in table 1. The general appearance of the water were clear, odour of the brackish and marine water have objectionable sensory evaluation.

Table 1: Physical and chemical characteristics of fresh, brackish and marine water.

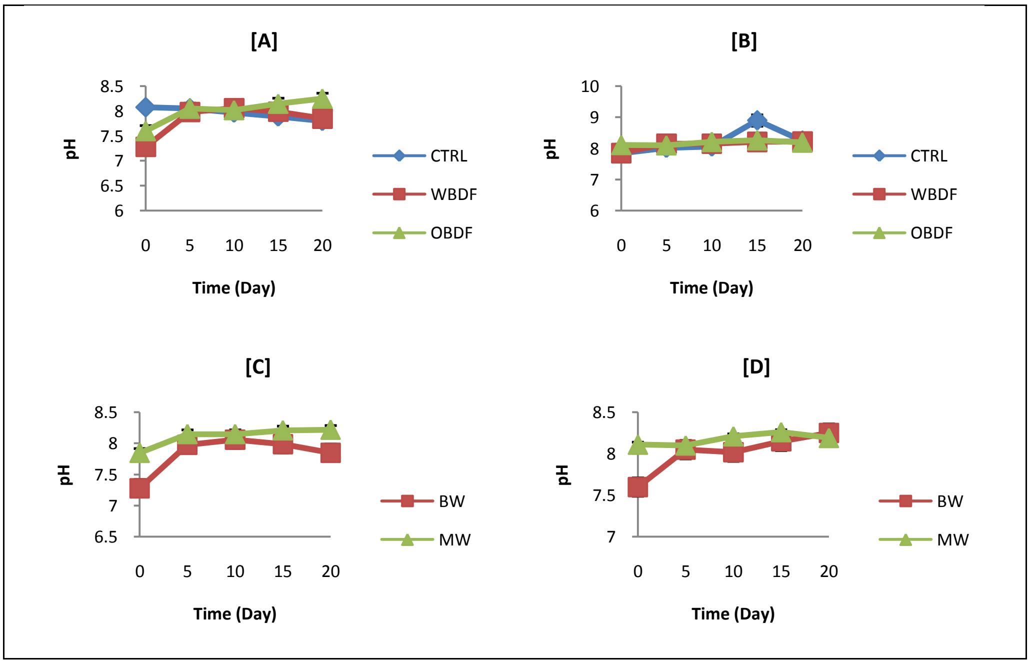
S/N	Physico-chemical characteristics	Unit	Brackish water	Marine water
1	General Appearance	-	Clear	Clear
2	Odour	-	Objectionable	Objectionable
3	Colour	Hazen	10.00	1.00
4	pH	-	7.96	8.21
5	Electrical Conductivity (EC)	µS/cm	7380.00	9200.00
6	Turbidity	NTU	2.00	<1.00

7	Total Hardness	mg/L	1920.00	3456.00
8	Total Alkalinity	mg/L	80.55	142.95
9	Chloride	mg/L	1568.55	3488.00
10	Total Suspended Solids (TSS)	mg/L	44.50	35.10
11	Total Dissolved Solids (TDS)	mg/L	6960.00	7400.00
12	Total Solids	mg/L	7004.50	7435.00
13	Nitrate	mg/L	1.65	1.90
14	Sulphate	mg/L	2.50	998.50
15	Calcium	mg/L	384.00	1152.00
16	Magnesium	mg/L	234.20	140.50
17	Biochemical Oxygen Demand (BOD)	mg/L	27.90	9.10
18	Chemical Oxygen Demand (COD)	mg/L	66.90	48.1
19	Total Iron (Fe)	mg/L	0.21	0.81
20	Lead (Pb)	mg/L	0.07	<0.001
21	Copper (Cu)	mg/L	<0.001	0.02

Colour decreases from 10.0 to 1.0 Hazen, pH increased/ranges from 7.96 to 8.21, Electrical conductivity (EC) ranges from 7380 to 9200µS/cm. Turbidity ranges from <1.0 to 2.0 NTU with marine water having the lowest and brackish water the highest. Total hardness ranges from 1920 to 3456 mg/L, Total alkalinity (80.55 – 142.95mg/L), Chloride (1568.55 – 3488.00 mg/L). Total Dissolved Solids followed a similar pattern ranging from 6960.0 to 7400.0 mg/L, Total Solids ranged from 7004.5 to 7435.0 mg/L but Total Suspended Solids ranged from 35.1 to 44.5 mg/L with brackish water having the highest value, marine water the lowest. The value of other parameters in brackish and marine water were as follows: Nitrate (1.65 and 1.90 mg/L), Sulphate (2.50 and 998.50 mg/L), Calcium (384.00 and 1152.00 mg/L), Magnesium (234.00 and 140.50 mg/L). Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) did not reflect this pattern; rather brackish water shows the highest value.

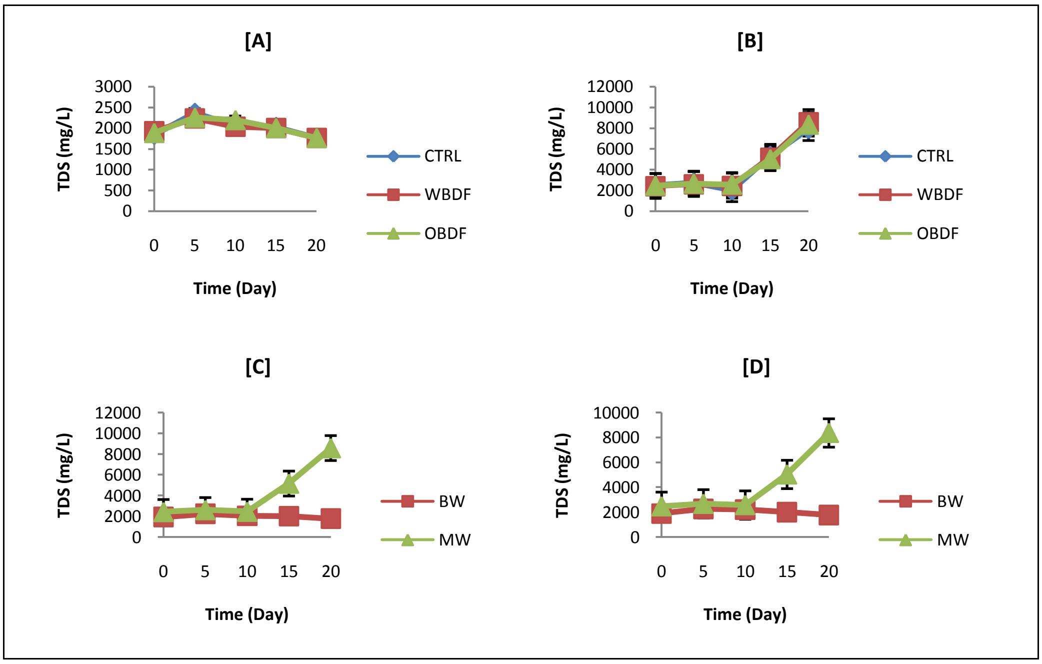
Physico-chemical result during biodegradation of drilling fluid in brackish and marine water

The values of the physico-chemical parameters: Hydrogen ion concentration [pH], Total Dissolved Solids [TDS], Dissolved Oxygen [DO], Biochemical Oxygen Demand [BOD], Chemical Oxygen Demand [COD], Ultimate Biodegradability from percentage ratio of Biochemical Oxygen Demand to Chemical Oxygen Demand taken during biodegradation of Drilling fluid - oil base and water base for day 0, 5, 10, 15and 20 in fresh, brackish and marine water were shown in Fig. 1 – 6 respectively.



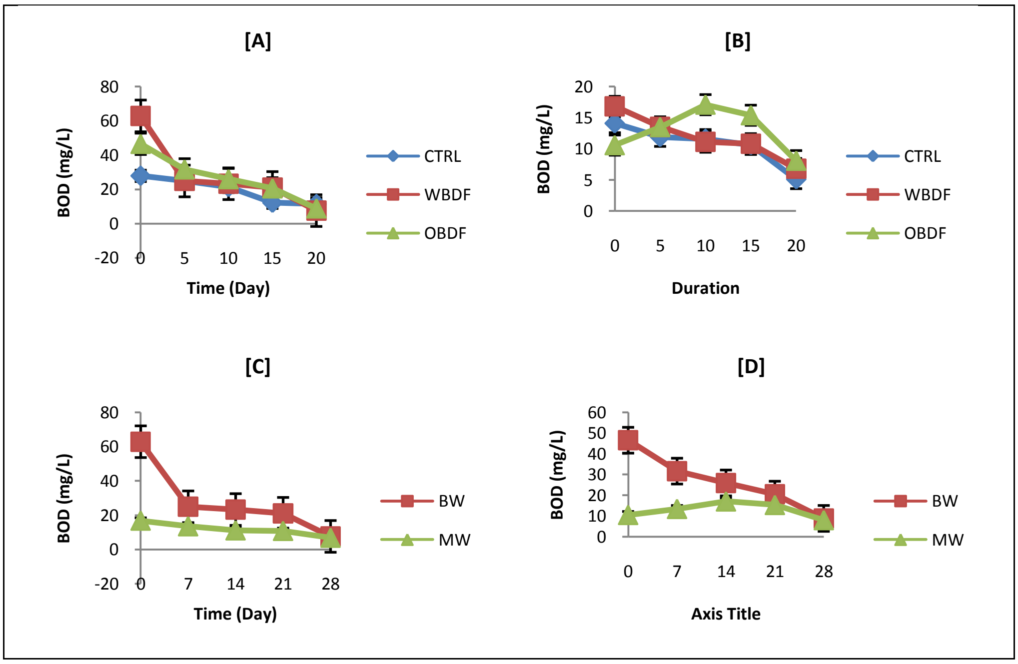
Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, pH = Hydrogen ion concentration*.

Fig. 1: [A] Changes in Hydrogen ion (pH) concentration during biodegradation of drilling fluid in Brackish water. [B] Changes in Hydrogen ion (pH) concentration during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Hydrogen ion (pH) concentration during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Hydrogen ion (pH) concentration during biodegradation of OBDF in brackish and marine ecosystem



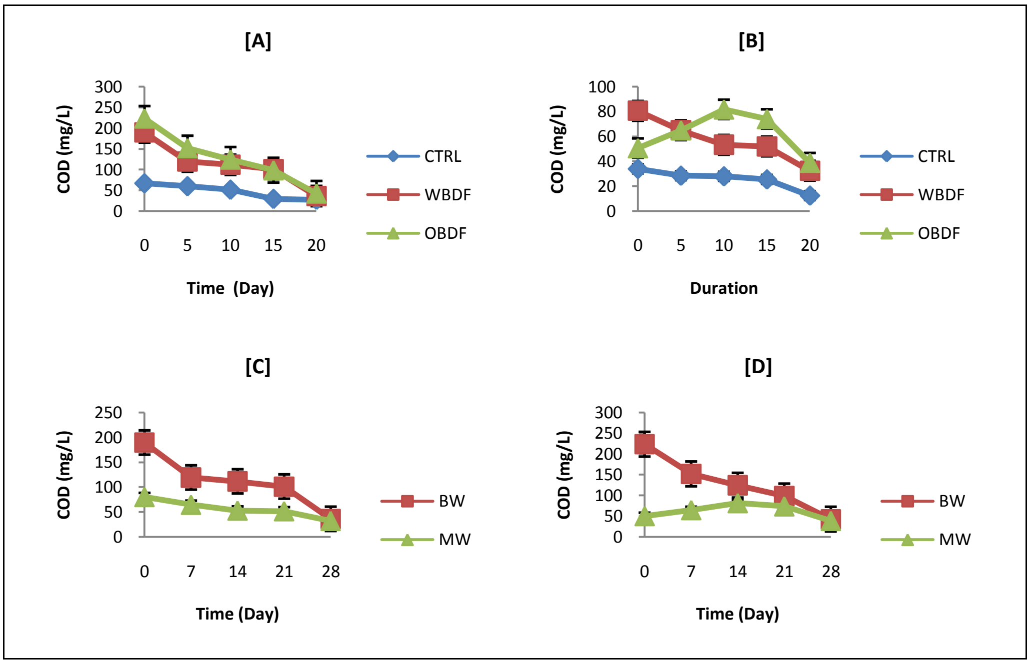
Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, TDS = Total Dissolved Solids*

Fig. 2: [A] Changes in Total Dissolved Solids (TDS – mg/L) during biodegradation of drilling fluid in Brackish water. [B] Changes in Total Dissolved Solids (TDS – mg/L) during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Total Dissolved Solids (TDS – mg/L) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Total Dissolved Solids (TDS – mg/L) during biodegradation of OBDF in brackish and marine ecosystem



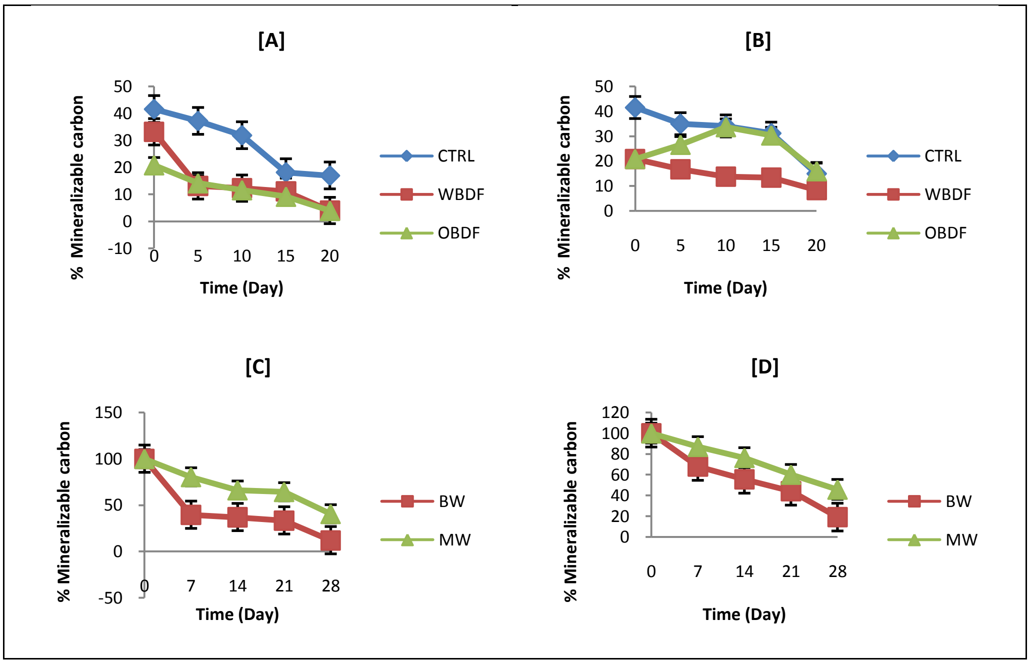
Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, BOD = Biochemical Oxygen Demand*

Fig. 3: [A] Changes in Biochemical Oxygen Demand (BOD –mg/L) during biodegradation of drilling fluid in Brackish water. [B] Changes in Biochemical Oxygen Demand (BOD –mg/L) during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Biochemical Oxygen Demand (BOD –mg/L) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Biochemical Oxygen Demand (BOD –mg/L) during biodegradation of OBDF in brackish and marine ecosystem



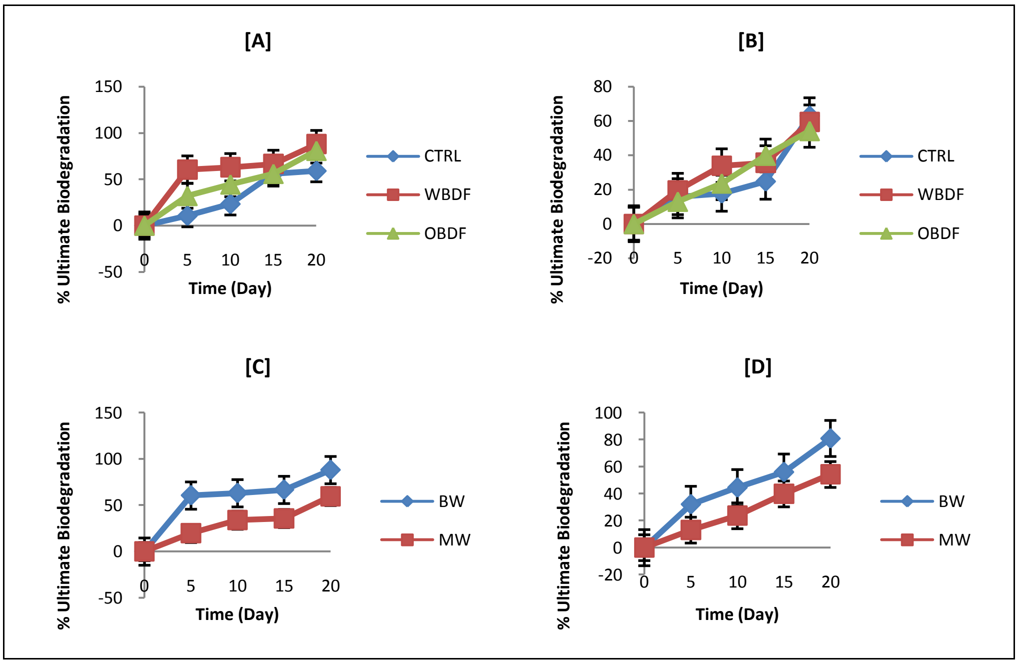
Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, COD = Chemical Oxygen Demand*

Fig. 4: [A] Changes in Chemical Oxygen Demand (COD – mg/L) during biodegradation of drilling fluid in Brackish water. [B] Changes in Chemical Oxygen Demand (COD – mg/L) during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Chemical Oxygen Demand (COD – mg/L) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Chemical Oxygen Demand (COD – mg/L) during biodegradation of OBDF in brackish and marine ecosystem



Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water,*

Fig. 5: [A] Changes in Percentage (%) Mineralizable carbon during biodegradation of drilling fluid in Brackish water. [B] Changes in Percentage (%) Mineralizable carbon during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Percentage (%) Mineralizable carbon during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Percentage (%) Mineralizable carbon during biodegradation of OBDF in brackish and marine ecosystem

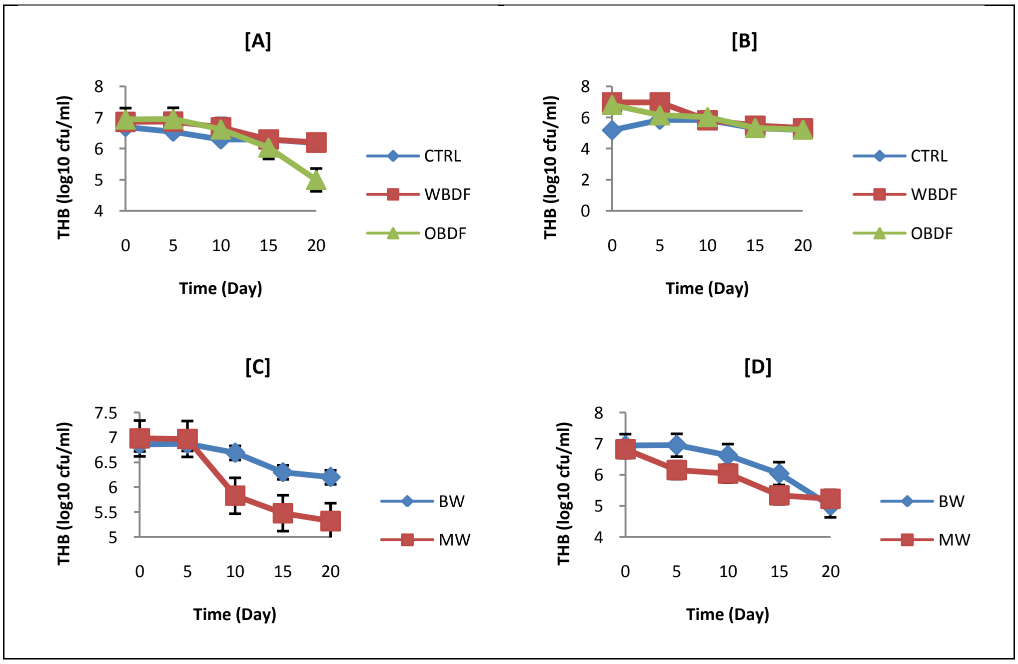


Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water,*

Fig. 6: [A] Changes in Percentage (%) Ultimate Biodegradation during biodegradation of drilling fluid in Brackish water. [B] Changes in Percentage (%) Ultimate Biodegradation during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Percentage (%) Ultimate Biodegradation during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Percentage (%) Ultimate Biodegradation during biodegradation of OBDF in brackish and marine ecosystem

Microbiological result during biodegradation of drilling fluid brackish and marine water

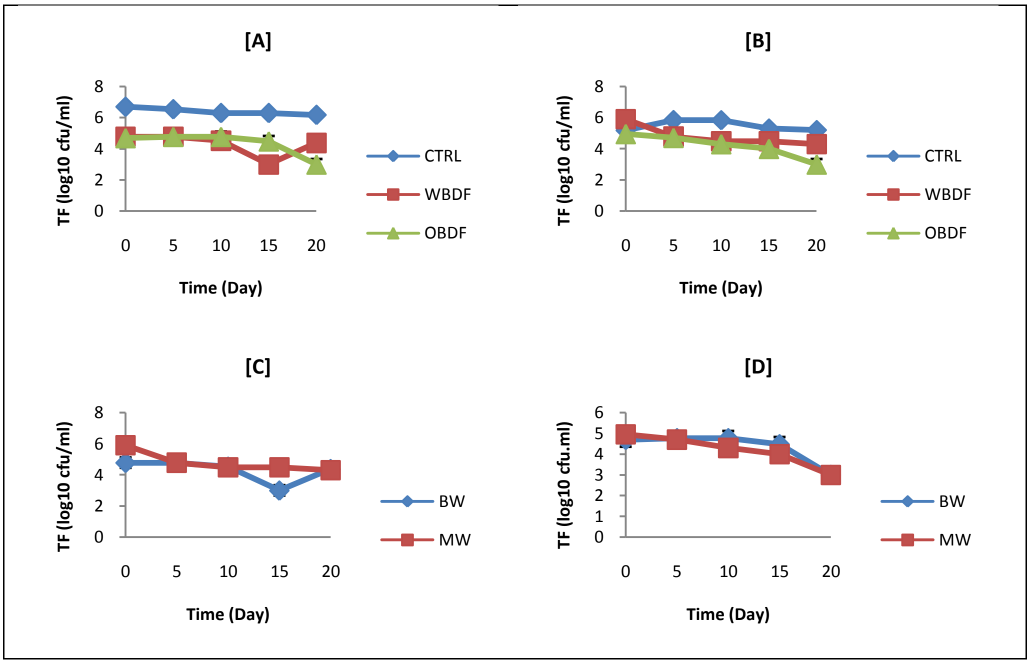
The values of microbiological parameters: Total Heterotrophic Bacteria [THB], Total Fungi [TF], Drilling Fluid Utilizing Bacteria [DFUB], taken during Biodegradation of Drilling fluid - oil base and water base for day 0, 5, 10, 15 and 20 in brackish and marine water were shown in Fig. 7-9.



Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, THB = Total Heterotrophic Bacteria*

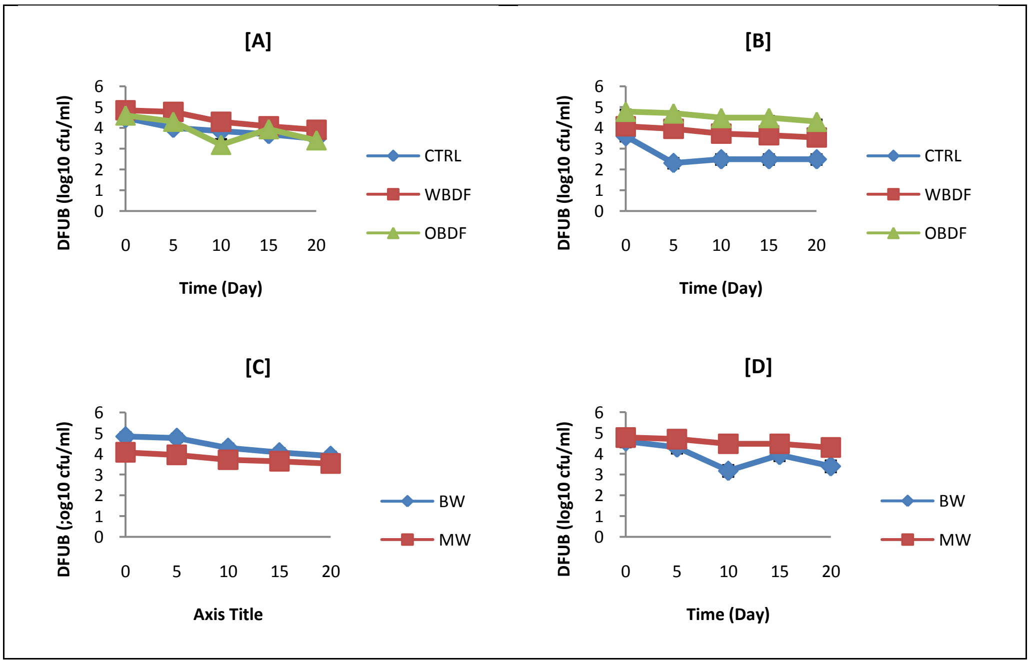
Fig. 7: [A] Changes in Total Heterotrophic Bacteria (THB – log10 cfu/ml) during biodegradation of drilling fluid in Brackish water. [B] Changes in Total Heterotrophic Bacteria (THB – log10 cfu/ml) during

biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Total Heterotrophic Bacteria (THB - log10 cfu/ml) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Total Heterotrophic Bacteria (THB - log10 cfu/ml) during biodegradation of OBDF in brackish and marine ecosystem



Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, TF = Total fungi*

Fig. 8: [A] Changes in Total Fungi (TF – log10 cfu/ml) during biodegradation of drilling fluid in Brackish water. [B] Changes in Total Fungi (TF – log10 cfu/ml) during biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Total Fungi (TF - log10 cfu/ml) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Total Fungi (TF - log10 cfu/ml) during biodegradation of OBDF in brackish and marine ecosystem



Key: *CTRL = Control, WBDF = Water base drilling fluid, OBDF = Oil base drilling fluid, BW = Brackish water, MW = Marine water, DFUB =Drilling fluid Utilizing Bacteria*

Fig. 9: [A] Changes in Drilling fluid Utilizing Bacteria (DFUB – log10 cfu/ml) during biodegradation of drilling fluid in Brackish water. [B] Changes in Drilling fluid Utilizing Bacteria (DFUB – log10 cfu/ml) during

biodegradation of drilling fluid in Marine water. [C] Comparative evaluation of Drilling fluid Utilizing Bacteria (DFUB - log10 cfu/ml) during biodegradation of WBDF in brackish and marine ecosystem. [D] Comparative evaluation of Drilling fluid Utilizing Bacteria (DFUB - log10 cfu/ml) during biodegradation of OBDF in brackish and marine ecosystem

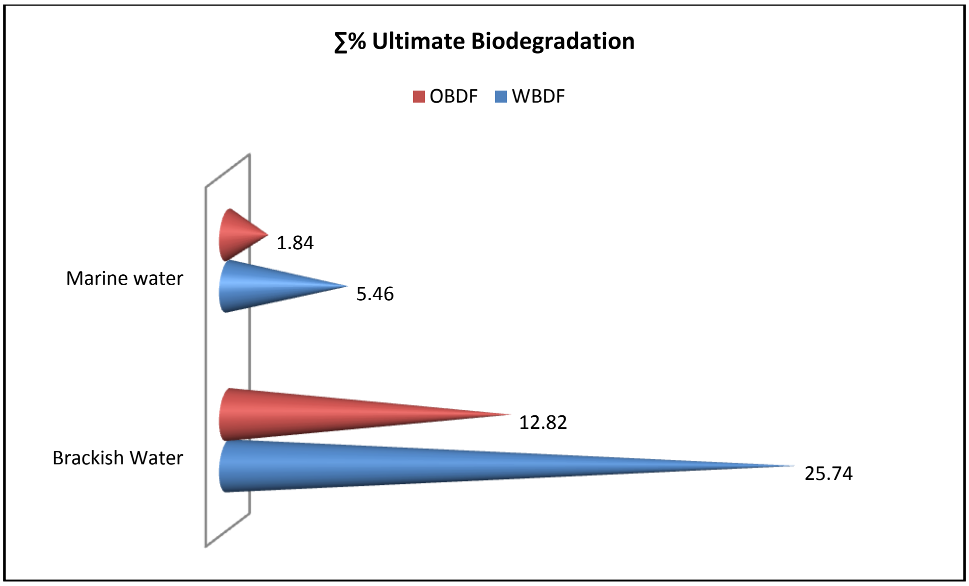


Fig. 10 Cumulative Percentage (∑%) Ultimate Biodegradation of Water base and Oil base Drilling Fluid in brackish and marine water

IV. Discussion

The results of some physical and chemical properties of the biodegradation set-up showed that differences in Hydrogen ion concentration (pH) and organic carbon (biodegradable and chemically oxidizable) content of the two aquatic systems (brackish and marine) were not statistically significant at 95% probability levels (Table 1). Thus the nutrient load of these aquatic systems did not offer any significant advantage to the indigenous microflora (Odokuma and Otukunafor, 2003). However, the alkaline pH ranges as well as mesophilic temperature range was observed to favour the acclimatization process for the native petroleum product-utilizing microbial population. These physic-chemical factors were particularly important for the survival of petroleum product-utilizing microbial consortium in the aquatic systems. These findings corroborated with the findings of Okpokwasili and Olisa (1991). With respect to sensory evaluation: General appearance of the brackish and marine water was clear; Odour of brackish and marine water was objectionable (Table 1). This could probably be due to oxidation of petroleum and other organic matter discharged on daily basis into the water body, since the aquatic area of study serve multiple purposes for oil and gas industry, manufacturing and fabrication industries etc. The Electrical Conductivity (EC), Total Hardness, Chloride, Total Dissolved Solids (TDS), Total Solids and Calcium of the three aquatic systems increased in the following order: Brackish water < Marine water while colour took the reverse order. Other parameters such as Total Alkalinity, Total Suspended Solids (TSS), Magnesium, Lead (Pb), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) showed an alternate trend: Brackish water > Marine water. This could be attributed to the presence and amount of organic matter present in the sample at the point of collection (Nrior *et al*., 2017b).

Ten bacterial isolates obtained from different mixture of petroleum products (Drilling fluid - oil base and water base) with the different water (brackish and marine) samples were identified to belong to the following genera; *Micrococus*, *Corynebacterium*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Proteus*, *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*. Total heterotrophic bacteria count decreases from day 1 to day 20; with water base drilling fluid (WBDF) having the highest cumulative count in brackish water(6.58±0.32 log10cfu/ml) and marine water (6.58±0.32) (Fig. 7). Statistical evaluation of the growth of THB result during the biodegradation of petroleum products revealed the following order: Total Heterotrophic Bacteria (THB - log10 cfu/ml) in brackish water: Water base (6.58±0.32) > Control (6.40±0.21) > Oil base (6.31±0.82). Total Heterotrophic Bacteria (THB – log10 cfu/ml) in marine water: Water base (6.12±0.81) > Oil base (5.92±0.65) > Control (5.47±0.34). Generally, this meant that the intermediates produced from the degradation of drilling fluids in these aquatic systems favoured the growth of a larger population of heterotrophic bacteria. Odokuma and Otukunafor (2003); Nrior, *et al.,* (2017a,b) observed a similar trend.

Six genera of fungal isolates identified were – *Aspergillus, Alternaria, Fusarium, Penicilium, Rhizopus* and *Mucor.* The Macroscopic morphology was based on best growth temperature, growth rate, colour on SDA, colour on reverse side, texture and special feature while the microscopic morphologies and identities of the different species of the fungal isolates based on characteristic features of conidiopore, phialides, vesicle, sclerotia, hulle cells, sporangiophore, apophysis, columella, sporangium and rhizoids. The fungal species identified were: *Aspergillus flavus, Aspergillus fumigates, Aspergillus versicolor, Penicilium marneffei, Penicilium chrysogenum, Rhizopus microspores, Rhizopus arrhizus, Mucor racemosus, Mucor amphibiorum.* The trend observed in THB was same with total fungal population in terms of growth with increased time duration. Statistical evaluation of the growth of total fungi (Fig. 8) during the biodegradation of petroleum products revealed the following; *Total Fungi (TF – log10 cfu/ml) in brackish water:* Control (6.40±0.21) > Oil base (4.34±0.76) > Water base (4.29±0.74). *Total Fungi (TF – log10 cfu/ml) in marine water:* Control (5.47±0.34) > Water base (4.79±0.65) > Oil base (4.19±0.76). These test drilling fluids showed mild increases and decreases in the total microbial (fungal) population in brackish and marine water used as inoculums. This observation is in agreement with the report of Okpokwasili and Nnubia (1995); Nrior and Wosa (2016) that, oil spill dispersants support mild increases (stimulation) and decrease (inhibition) in the growth of specific heterotrophic marine bacteria. This response also applies to fungal population in this study.

Acclimatization of the microbial population with petroleum product components enhances the biodegradation efficiency of the microorganisms. Although bacterial population was more than fungal drilling fluids degraders in the three aquatic systems, this agrees with previous findings of Okpokwasili and Olisa (1991); Amund *et al.* (1997). The adaptability of native microbial population in the three aquatic systems to petroleum products components would be the reason for their success at mineralizing the petroleum products in the experimental set-up where the physic-chemical properties of the ecosystem were supportive to the survival of these microorganisms (Spain and van Veld, 1983; Nrior and Odokuma, 2015). Evaluation Drilling fluid utilizing bacteria (DFUB) in the two aquatic systems (Fig. 9) revealed their population as follows: Drilling fluid Utilizing Bacteria (DFUB - log10 cfu/ml) in brackish water: Water base (4.38±0.42) > Control (3.90±0.38) > Oil base (3.88±0.60); Drilling Fluid Utilizing Bacteria (DFUB – log10 cfu/ml) in marine water: Oil base (4.55±0.19) > Water base (3.79±0.22) > Control (2.67±0.53)

The study revealed genera of *Pseudomonas, Bacillus, Proteus, Micrococcus, Rhizopus, Aspergillus and Penicilium* isolated from brackish and marine ecosystems were capable of utilizing both drilling fluid (Water base and Oil base) as their carbon source. Similar trend in the ability of natural microbiota to degrade novel or synthetic compounds has been reported (Green, 1994; Ogbulie *et. al*., 2008; Nrior and Wosa, 2016; Nrior, *et al* 2017a,b). Such similarity in the utilization of novel compounds by natural microflora is expected, since such breakdown depends on the possession of plasmids that are not naturally present in all microorganisms (Ogbuilie *et. al.,* 2008). Hence, the ability to utilize xenobiotics must be dependent on the possession of the requisite enzymes necessary for such degradation.

Ultimate biodegradability two types of Drilling fluid – water base drilling fluid (WBDF) and oil base drilling fluid (OBDF) used in the upstream sector of the Nigeria petroleum industry investigated in brackish and marine ecosystems shows cumulative percentage (∑%) ultimate biodegradation of drilling fluid in brackish water were; WBDF (25.74), OBDF (12.82), while in marine water, WBDF (5.46), OBDF (1.84) (Fig. 10). Analysis of comparative percentage ultimate biodegradation rate revealed that WBDF is 2.01 times more biodegradable than OBDF in brackish water environment [WBDF 66.75%, OBDF 33.25%] whereas in marine ecosystem WBDF is 2.97 times more biodegradable than OBDF [WBDF 74.79%, OBDF 25.21%]. It further shows WBDF having higher differential rate of biodegradability in marine than brackish (that is, WBDF is more readily biodegradable than OBDF in marine water than brackish water).Drilling fluid utilizing bacteria genera isolated were; *Pseudomonas, Bacillus, Micrococcus, Enterobacter* and fungi; *Aspergillus, Penicillium, Rhizopus* and *Mucor.*

V. Conclusion

Physicochemical characteristics of the two aquatic systems (Brackish water from Trans-Amadi Industrial Layout River and Marine water from Bonny River) were heavily impacted with toxic substances from oil companies and industries constantly using these water environments for discharge of effluents and other petroleum products in Rivers State, Nigeria. Also, the same specie of bacteria and fungi from different aquatic ecosystems could respond differently to the same level of a toxicant (such as drilling fluid) from time to time even when all other variables are held constant.

Analysis of comparative percentage ultimate biodegradation rate revealed that WBDF is 2.01 times more biodegradable than OBDF in brackish water environment whereas in marine ecosystem WBDF is 2.97 times more biodegradable than OBDF..Drilling fluid utilizing bacteria genera isolated were; *Pseudomonas, Bacillus, Micrococcus, Enterobacter* and fungi; *Aspergillus, Penicillium, Rhizopus* and *Mucor.* In view of these findings, water base drilling fluid should be adopted as best option for drilling operations in both brackish and marine water environment in Nigeria due to its high level of eco-friendliness both ecosystem in comparison to OBDF.

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