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Enhanced biodegradation of water-based drilling fluids in marine water

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ABSTRACT

Drilling fluids/mud is an essential component of the rotary drilling process used to lubricate and cool down the drilling bit during the operation of oil and gas on land and in offshore environments. Disposing of drilling fluids into marine water ecosystems can lead to pollution, affecting aquatic life and water quality. Enhanced biodegradation of drilling fluids: water-base drilling fluid (WBF) in marine water (MW) using different bioaugmenting organisms (*Pseudomonas aeruginosa* strain EB38 and *Bacillus safensis* strain UIS0051) and biostimulating agent: fish waste (FW) was investigated. The rate of biodegradation was estimated from the percentage (%) reduction of Total Hydrocarbon Content (THC) with percentage (%) efficiency of bioaugmenting organisms; *Pseudomonas* (Pse), *Bacillus* (Bac) and biostimulating agent; fish waste (FW) from day 1 to the last day (28). The study employed a Randomized Complete Block Design (RCBD) with 15 experimental setups, each with a final volume of 1000 ml. After the contamination, bioaugmenting organisms and biostimulating agents were applied. Some physicochemical and microbiological parameters of the water sample were determined before and after contamination. Physicochemical parameters monitored were temperature, pH, Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Electrical Conductivity (EC), Biochemical Oxygen Demand (BOD), Nitrate, Phosphate, Sulphate, Chemical Oxygen Demand (COD) Total Hydrocarbon Content (THC), while microbiological characteristics were total heterotrophic bacteria (THB), total fungi count (TFC), Drilling Fluids Utilizing Fungi (DFUF) and Drilling Fluids Utilizing Bacteria (DFUB). From the initial THC contamination value of 3282.7mg/L (MW+WBF), the amount remediated and % biodegradation of THC at 28 days in the different treatment set up in decreasing order is as follows: MW+WBF+Bac (3236.67mg/L; 98.60%) > MW+WBF+FW+Bac (3224.67 mg/L; 98.23%) > MW+WBF+Pse (3198.67mg/L; 97.32%) > MW+WBF+FW+BAC+PSE (3186.67mg/L; 97.08%) > MW+WBF+FW+PSE (3174mg/L; 96.69%) and MW+WBF+FW (3123.34mg/L; 95.15%). The results suggest that microbial inoculants and fish waste can be effective amendments for enhancing the biodegradation of THC in drilling fluids, with potential applications in environmental remediation.

Keywords: Drilling fluid, marine water, bioaugmenting organisms, fish waste.

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INTRODUCTION

The oil and gas drilling operations generate significant waste, including water-based drilling fluids (WBDFs) and drill cuttings (Neff et al., 2000). WBDFs, comprising water, clay, polymers, and additives, can become spent and transform into hazardous waste products, posing environmental and public health concerns due to their potential toxicity and persistence (Sharif et al., 2017). The discharge of spent drilling muds and cuttings can contaminate aquatic and terrestrial ecosystems, causing harm to living organisms and affecting soil quality, water, and the entire ecosystem (Sharif et al., 2017). Inadequate management of drilling waste can lead to severe environmental consequences. Regulatory agencies, such as the US Environmental Protection Agency (EPA),

oversee the discharge of drilling muds and cuttings into federal and state waters, enforcing restrictions outlined in the Effluent Limitation Guidelines (Neff et al., 2000). Drilling operations produce two primary waste streams: drilling fluid waste and drill cuttings, typically disposed of through offshore disposal, onshore disposal, or drill cutting re-injections (Neff et al., 2000). To alleviate the environmental impact of drilling waste, various treatment and disposal options are employed, including underground injection, land application, biological processes, and thermal treatment (Furukawa et al., 2017). Drilling fluids containing heavy metals and hydrocarbons can cause DNA damage, mutations, cancer, and other harmful health effects in humans (Adewole et al., 2010; Jadoon and Malik, 2017). The release of drilling fluids containing heavy metals into aquatic environments can have severe consequences, including bioaccumulation and biomagnification of heavy metals in the food chain (Jaishankar et al., 2014), toxicity to aquatic organisms (DesMarias and Costa, 2019), alterations in aquatic ecosystems (Ayotamuno et al., 2009), and damage to habitats and ecosystems (Geier *et al.,* 2015), ultimately leading to reproductive and developmental toxicity (Fasinu and Orisakwe, 2013), neurotoxicity (Jadoon and Malik, 2017), and immune system suppression (DesMarias and Costa, 2019) in aquatic organisms. Bioremediation, a cost-effective and eco-friendly technology, offers a promising solution for detoxifying complex mixtures of drilling fluids and reducing their environmental footprint (Sharif et al., 2017). This study focuses on enhancing the biodegradation of water-based drilling fluids in marine water, exploring the potential of biostimulation and bioaugmentation to minimize the environmental impacts of drilling waste.

MATERIALS AND METHODS

Description of the study area

The marine water used for the experiment was obtained from the Bonny River. Bonny River is between latitude 4°28’06.2” N and longitude 7°08’20.0” E. Bonny River is a hub of Nigeria's oil and gas industry. It encompasses an Island called Bonny Island. Bonny Island hosts several major industrial facilities, including the Nigeria liquefied natural gas (LNG) terminal.

The river is crucial for transporting petroleum products and serves as a conduit for export activities. Many local inhabitants engage in fishing, agriculture, and related activities, relying on the river's resources for sustenance and economic activity. Industrial activities, particularly oil exploration and production, have led to significant pollution in the river. Oil spills, industrial waste disposal, and other contaminants significantly endanger water quality and aquatic life.

Sample collection and preparation of the marine water

Marine water was obtained from Bonny River with protective equipment (hand gloves) to avoid contamination. A sterile sample bottle and cap were rinsed with the river water three times before collecting the sample. The bottles were submerged below the water's surface, with the mouth of the bottle opened facing upstream and then filled. All samples were kept in an ice-packed cooler after collection and were immediately transported to the Rivers State University Microbiology Laboratory for analysis within 24 hours of collection (APHA 2005).

Drilling fluids

Drilling fluids (water base) used in this experiment were obtained from Noble Gerry De Souza Rig, Nigeria. The fluids were collected in a sterile bottle and promptly transported to the Rivers State University Microbiology Laboratory for analysis.

Fish waste

Fish waste used as an organic nutrient for biostimulants was obtained from a cold room, located at Rumuokoro, Obio Akpor, Port Harcourt Rivers State in a sterile sample container and placed in an ice-packed cooler and immediately transported to Rivers State University Microbiology Laboratory for analysis within 24hrs of collection. The fish waste obtained was blended and sieved to ensure uniformity and purity of the material. Following this preparation, the liquid fraction was extracted.

Determination of physiochemical properties of the marine water sample

The marine water sample was analyzed before treatment and after treatment for temperature °C, hydrogen ion, pH, total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrate, phosphate, sulphate, chemical oxygen demand (COD), chlorine, bromine, alkalinity, hardness, salinity, total solids, turbidity, total hydrocarbon content (THC), and microbiological parameters following standard procedures as described by APHA (2012).

Microbiological Analysis of samples

Enumeration of total heterotrophic bacteria (THB)

A ten-fold serial dilution was carried out by measuring

1ml of a water sample using a pipette and was transferred aseptically into a sterile test tube containing 9ml of normal saline using the spread plate method described by Prescott et al. (2002). Aliquots of 0.1ml water sample from 10^{-6} test tubes were inoculated into solidified nutrient agar in triplicate. The culture plates were incubated invertedly at 30^{o}C for 24 hours. Discrete colonies developed were counted and expressed in CFU/ml (Ibiene et al., 2011).

Enumeration of total fungi (TFC)

A ten-fold serial dilution was carried out by measuring 1ml of a water sample using a pipette and was transferred aseptically into a sterile test tube containing 9ml of normal saline using the spread plate method described by Prescott et al. (2005). Aliquots of 0.1ml water sample from 10^{-4} test tubes were inoculated into solidified SDA in triplicate. The culture plates were incubated invertedly for 3-5days at room temperature (28°C). Discrete colonies developed were counted and expressed in CFU /ml (Morikawa et al., 2000).

Enumeration and isolation of drilling fluids utilizing bacteria (DFUB)

The culture medium used to isolate drilling fluids utilizing bacteria was mineral salt agar (MSA). Drilling fluids utilizing bacteria (DFUB) were isolated and enumerated using the vapour phase transfer method as adopted by Ibiene et al. (2011). An aliquot of 0.1ml of the 10^{-2} dilutions was plated onto solidified MSA plates medium supplemented with nystatin to inhibit the growth of fungi, and the plates were inverted. Filter papers were placed inside the cover of the inverted plates and flooded with 1ml of drilling fluids as a source of carbon and energy. The plates were incubated at 37^{0}C for 5 to 7 days. Discrete colonies that developed were counted and expressed in CFU/ml. To obtain a pure culture, the bacterial isolates were sub-cultured using the accepted techniques, and the pure cultures were characterized and identified to ascertain the bacterial species.

Enumeration and isolation of drilling fluids utilizing fungi (DFUF)

Drilling fluids utilizing fungi (DFUF) were isolated and enumerated using the vapour phase transfer method as adopted by Ibiene et al. (2011). An aliquot of 0.1ml of the 10^{-2} dilutions was inoculated onto solidified MSA plates medium supplemented with chloramphenicol (antibiotics) to inhibit the growth of bacteria, and the plates were inverted. Filter papers were placed inside the cover of the inverted plates and flooded with 1ml of drilling fluids as a source of carbon and energy. The plates were incubated at 28^{0}C for 5 to 7 days. Discrete colonies that developed were counted and expressed in CFU/ml. To obtain a pure culture, the fungal isolates were sub-cultured using the accepted techniques, and the pure cultures were characterized and identified to ascertain the fungi species.

Identification and characterization of bacterial and fungal isolates

Each isolate obtained after isolation was characterized based on colonial, microscopic, and macroscopic morphology. Bacterial colonies were characterized based on their colour, form, elevation, size, and margin. Fungal growth on plates was characterized based on form, colour, was observed, surface, spore, and underside colour. A smear was made on a slide and stained with lactophenol cotton blue. The slides were examined using a microscope (x40) for the structure of hyphae and details of the sporulation structure. Discrete bacterial and fungal colonies were purified through subculturing. The following sets of biochemical tests were used to characterize the pure colonies. The tests include Gram reaction and sugar fermentation, catalase, motility, oxidase, indole, citrate, and methyl red-Voges-Proskaver (MR-VP). The isolates were identified according to descriptions in the Bergeys Manual of Determinative Bacteriology (Holt, 2000) and recorded accordingly.

Preparation of bacterial suspension for biodegradation setup

Suspension of *Pseudomonas and Bacillus sp* were prepared from a 24-hour pure culture Petri dish as described by Hadibarata and Tachibana (2009). Two hundred millilitres (200 ml) of nutrient media broth were transferred into a 250 ml conical flask and was sterilized using an autoclave at 121^{o}C for 15 minutes at 15psi and allowed to cool at room temperature. Zero-point eight gram (0.8g) of Nystatin was supplemented to the broth. *Pseudomonas sp* were scraped out from the surface of bacterial colonies and then transferred to the 250 ml flask containing the 200 ml nutrient broth until a turbid suspension was formed. This was incubated at room temperature (28^{o}C) for 48hrs.

Biodegradation studies

The biodegradation experimental setup as shown in Table 1, consists of eight treatments (T1-T8) designed to evaluate the effects of various bio-augmenting organisms and bio-stimulating agents on the degradation of water-

Table 1. Biodegradation experimental set-up.

Treatment code		Marine water	Water based	Bac	Pse	Fish waste	Final volume
T1	UW	-	-	-	-	-	1000
T2	MW+WBF	990	10	-	-	-	1000
T3	MW+WBF+Bac	940	10	50	-	-	1000
T4	MW+WBF+Pse	940	10	-	50	-	1000
T5	MW+WBF+FW	950	10	-	-	40	1000
T6	MW+WBF+FW+Bac	900	10	50	-	40	1000
T7	MW+WBF+FW+Pse	900	10	-	50	40	1000
T8	MW+WBF+FW+Bac+Pse	900	10	25	25	40	1000

KEY: UW - Uncontaminated Water, **MW** - Marine Water, **WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **BAC** - *Bacillus,* ***Pse*** - *Pseudomonas.*

based drilling fluid (WBF) in marine water. Treatment 1 (T1) serves as a negative control, comprising only uncontaminated water. In contrast, Treatments 2-8 involve marine water contaminated with 10 mL of WBF and varying combinations of bio-augmenting organisms and bio-stimulating agents. The bio-augmenting organisms used are *Bacillus* (Bac) and *Pseudomonas* (Pse), added in 50 mL volumes to specific treatments (T3, T6, T8 for Bac and T4, T7, T8 for Pse). Fish waste (FW) is used as a bio-stimulating agent, added in 40 mL volumes to Treatments 5-8. Each treatment has a final volume of 1000 mL, comprising marine water (MW) volumes ranging from 940-990 ml. To ensure homogeneous mixing and optimal contact between microorganisms and contaminants, the water was stirred periodically throughout the experimental period. The following physicochemical and microbiological parameters were analyzed: dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrate, phosphate, sulphate, chemical oxygen demand (COD), total hydrocarbon content (THC), total heterotrophic bacteria (THB), total fungi (TF), drilling fluids utilizing bacteria (DFUB) and drilling fluids utilizing fungi (DFUF) in triplicates, and were monitored for 28 days at 14 days intervals.

Percentage (%) biodegradation evaluation

The percentage (%) of biodegradation was calculated from the formula used by Nrior et al. (2017b) as follows:

Step 1: Amount of pollutant degraded equals to Initial concentration of pollutant (Day 0) minus the final concentration of pollutant at the end of the experiment (last day).

Step 2: Percentage (%) biodegradation equals to amount of pollutant remediated divided by initial concentration of pollutant (Day 0 or 1) multiplied by 100.

Thus;

BC = IC – FC
Bx = IC – I0

Were,

BC = Amount of pollutant degraded
IC = Initial concentration of pollutant (Day 0)
FC = Final concentration of pollutant at end of experiment (Last day)
I0 = Initial concentration value of Control at day 0
Bx = Actual amount of pollutant in test medium

% Biodegradation = BC x 100 Bx Eqn (1)

Efficiency biodegradation

The efficiency of biodegradation was calculated using the;

Efficiency of enhancer = amount remediated by each enhancer - amount remediated by Indigenous microorganisms in control (contaminant in water only)

Percentage efficiency

Percentage efficiency was calculated using the;

Efficiency by each enhancer / total efficiency ×100

Statistical analysis

Data obtained from the biodegradation experimental setup were subjected to statistical analysis using a computer-based program. SPSS version 22 for Analysis of Various (ANOVA) and Excel on Microbiological, Total hydrocarbon content (THC), and physicochemical parameters to compare data between marine water in all treatments and controls and test whether the different nutrient amendments given to the drilling fluids

contaminated with marine water were significant at a confidence level of 95% or P<0.05. The results were expressed as Mean ± Standard Deviation.

RESULTS

Physicochemical and microbiological properties of the water before application of the various treatments

The marine water sample has a temperature of 30°C and a neutral pH of 7.6. It has high total dissolved solids (59330 mg/L) and electrical conductivity (12300 µS/cm), indicating a high concentration of dissolved substances. The total suspended solids are very low (0.01 mg/L), and chlorine and bromine are undetectable (<0.01 mg/L). The water has high alkalinity (3322.48 µeq/L) and hardness (4033.43 mg/L) but low salinity (7.5 mg/L). The turbidity was very low (0.013 NTU), and the dissolved oxygen level was low (2 mg/L). The biochemical oxygen demand (1.94 mg/L) and chemical oxygen demand (3.41 mg/L) are also low. Nutrient levels, including nitrate (1.982 mg/L), sulphate (0.929 mg/L), and phosphate (0.231 mg/L), are low. However, the total hydrocarbon content was high (220 mg/L). The water was clear and had no unpleasant odour (Table 2).

The marine water sample contains a significant population of total heterotrophic bacteria, with a count of 1.45 ± 0.06 x 10^9 CFU/mL. The total fungi count is also notable, at 1.45 ± 0.47 x 10^5 CFU/mL. Additionally, the sample contains drilling fluids utilizing bacteria, with a count of 1.07 ± 0.08 x 10^5 CFU/mL, and drilling fluids utilizing fungi, with a count of 5.67 ± 0.58 x 10^3 CFU/mL (Table 3). These microorganisms indicate that the water sample has the potential for biodegradation of drilling fluids. The presence of these microorganisms suggests that the water sample may be capable of breaking down and utilizing the drilling fluids.

Table 2. Physicochemical characteristics of the marine water sample.

Parameter	Unit	Result
Temperature	°C	30
pH	-	7.6
Total Dissolved Solids (TDS)	mg/L	59330
Electrical Conductivity (EC)	µS/cm	12300
Total Suspended Solids (TSS)	mg/L	0.01
Chlorine	mg/L	<0.01
Bromine	mg/L	<0.01
Alkalinity	µeq/L	3322.48
Hardness	mg/L	4033.43
Salinity	mg/L	7.5
Turbidity	NTU	0.013
Dissolved Oxygen (DO)	mg/L	2
Biochemical Oxygen Demand (BOD)	mg/L	1.94
Chemical Oxygen Demand (COD)	mg/L	3.41
Nitrate	mg/L	1.982
Sulphate	mg/L	0.929
Phosphate	mg/L	0.231
Total Hydrocarbon Content (THC)	mg/L	220
Colour	-	Clear
Odour	-	Unobj.

Key: Unobj. = Unobjectionable to smell.

Table 3. Microbiological characteristics of the marine water sample.

Microbiological	Units	Marine water
THB	10^9CFU/ml	1.45±0.06
TFC	10^5CFU/ml	1.45±0.47
DFUB	10^5CFU/ml	1.07±0.08
DFUF	10^3 CFU/ml	5.67±0.58

Key: THB - Total heterotrophic bacteria, **TFC** - Total fungi count, **DFUB** - Drilling Fluids Utilizing Bacteria, **DFUF** - Drilling Fluids Utilizing Fungi.

Physicochemical and microbiological properties of fish waste before application for biodegradation analysis

The fish waste exhibited a temperature of 31.83°C and a slightly acidic pH of 6.28. The high electrical conductivity (13150 µS/cm) and total dissolved solids (65780 mg/L) indicate a highly concentrated solution. Moderate levels of organic pollution were observed, with a biochemical oxygen demand (BOD) of 4.6 mg/L and chemical oxygen demand (COD) of 8.11 mg/L. Nutrient levels were relatively low, with nitrate (12.3 mg/L), phosphate (0.38 mg/L), and sulphate (0.05 mg/L) present in limited quantities. However, high levels of total hydrocarbon content (192.67 mg/L) were detected, posing potential environmental concerns. Microbial analysis revealed moderate total heterotrophic bacteria (THB) counts (1.21±6.56 cfu/mL) and relatively high total fungi count (TFC) (6.67±1.53 cfu/mL) (Table 4).

Biochemical and morphological characteristics of the isolates

Bacterial species such as *Pseudomonas* sp, *Bacillus* sp, *Proteus* sp, and *Micrococcus* sp, as well as fungal genera including *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Candida* sp., and *Saccharomyces* sp., were isolated from marine water before and after contamination with drilling fluids.

Table 4. Fish waste profile.

Parameter	Units	Results
Temperature	°C	31.83
pH	-	6.28
Total Dissolved Solids (TDS)	mg/L	65780
Electrical Conductivity (EC)	µS/cm	13150
Dissolved Oxygen (DO)	mg/L	12.33
Biochemical Oxygen Demand (BOD)	mg/L	4.6
Chemical Oxygen Demand (COD)	mg/L	8.11
Nitrate	mg/L	12.3
Phosphate	mg/L	0.38
Sulphate	mg/L	0.05
Total Hydrocarbon Content (THC)	mg/L	192.67
Total Heterotrophic Bacteria (THB)	cfu/mL	1.21±6.56
Total Fungi Counts (TFC)	cfu/mL	6.67±1.53

Physicochemical characteristics of the water sample during biodegradation monitoring

The temperature ranged from 29.80°C in the UW treatment to 30.37°C in the MW+WBF+FW+Pse treatment. The pH levels ranged from 6.92 in the MW+WBF treatment to 7.20 in the MW+WBF+FW+Bac+Pse treatment. Total Dissolved Solids (TDS) increased from 26465 mg/L in the UW treatment to 65577 mg/L in the MW+WBF+FW+Bac treatment. Electrical Conductivity (EC) values ranged from 12590 µS/cm in the UW treatment to 48950 µS/cm in the MW+WBF treatment. Dissolved Oxygen (DO) levels ranged from 2.08 mg/L in the UW treatment to 5.32 mg/L in the MW+WBF+FW+Pse treatment. Biological Oxygen Demand (BOD) values increased from 0.76 mg/L in the UW treatment to 3.44 mg/L in the MW+WBF+FW+Bac+Pse treatment (Table 5a).

Nitrate levels ranged from 0.46 mg/L in the UW treatment to 3.89 mg/L in the MW+WBF+FW treatment. Phosphate levels ranged from 0.01 mg/L in the UW and MW+WBF treatments to 0.23 mg/L in the MW+WBF+FW and MW+WBF+FW+Pse treatments. Sulphate levels remained relatively consistent across treatments, ranging from 0.23 to 0.27 mg/L. Chemical Oxygen Demand (COD) levels ranged from 1.22 mg/L in the MW+WBF treatment to 6.32 mg/L in the MW+WBF+FW+Pse treatment. Total Hydrocarbon Content (THC) levels ranged from 124.44 mg/L in the UW treatment to 1966.89 mg/L in the MW+WBF treatment (Table 5b).

The biodegradation of total hydrocarbon content (THC) was observed in all treatments over 28 days. The highest percentage of bioremediation was achieved in Treatment T3 (MW+WBF+Bac) at 98.6%, followed closely by T6 (MW+WBF+FW+Bac) at 98.23%. The treatments with the highest amount of THC remediated were T3 (MW+WBF+Bac) at 3236.67 mg/L and T6 (MW+WBF+ FW+Bac) at 3224.67 mg/L. The control treatment (T1, UW) showed minimal biodegradation, with only 214.67 mg/L of THC remediated. The addition of microbial inoculants (*Bacillus safensis* and *Pseudomonas aeruginosa*) and fish waste (FW) enhanced the biodegradation of THC, with treatments T3, T4, T5, T6, T7, and T8 showing significant reductions in THC levels (Table 6).

The result (Figure 1) shows that the addition of

Table 5a. Mean and standard deviation of physicochemical parameters during enhanced biodegradation of drilling fluids in marine water.

Treatment code	Temperature (°C)	pH	Total Dissolved Solids (mg/l)	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/l)	BOD (mg/l)
UW	29.80±0.4a	7.00±0.85a	26465±36152.37a	12590.00±1587.67a	2.08±0.78a	0.76±0.57a
MW+WBF	29.90±0.46a	6.92±0.47a	58743±12221.51a	48950.00±42747.69a	1.20±0.56a	0.63±0.55a
MW+WBF+Bac	30.11±1.14a	7.01±0.26a	62180±15049.12a	12190.00±2592.93a	2.47±3.00a	1.90±3.09a
MW+WBF+Pse	29.92±1.12a	6.98±0.37a	58270±17990.47a	36520.00±39686.13a	2.84±4.07a	2.58±4.12a
MW+WBF+FW	29.96±1.07a	7.13±0.03a	61270±13117.43a	40623.33±45891.04a	3.87±5.29a	2.76±4.43a
MW+WBF+FW+Bac	29.60±1.22a	7.20±0.04a	65577±13824.41a	39590.00±42800.03a	4.64±6.03a	3.14±4.90a
MW+WBF+FW+Pse	30.37±0.93a	7.16±0.07a	51943±41927.85a	12588.67±4593.05a	5.32±6.28a	3.59±5.37a
MW+WBF+FW+Bac+Pse	30.30±1.21a	7.17±0.10a	50927±41605.76a	12640.00±4719.19a	5.09±5.62a	3.44±4.70a

Key: UW - Uncontaminated Water, **MW** - Marine Water, **WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051, ***Pse*** - *Pseudomonas aeruginosa EB38*, **BOD** - Biological oxygen demand.

Table 5b. Mean and standard deviation of physicochemical parameters during enhanced biodegradation of drilling fluids in marine water.

Treatment Code	Nitrate (mg/l)	Phosphate (mg/l)	Sulphate (mg/l)	COD (mg/l)	THC (mg/l)
UW	0.46±0.40f	0.01±0.00c	0.24±0.20a	1.66±0.45a	124.44±113.27a
MW+WBF	0.61±0.62ef	0.01±0.00c	0.24±0.21a	1.22±0.87a	1966.89±1141.96a
MW+WBF+Bac	1.04±0.51cdef	0.02±0.02c	0.24±0.20a	3.34±5.43a	959.78±1512.25a
MW+WBF+Pse	2.35±2.72bcdef	0.03±0.01c	0.25±0.20a	4.53±7.24a	820.67±1195.31a
MW+WBF+FW	3.89±2.12abc	0.23±0.08a	0.27±0.21a	4.86±7.79a	838.44±1151.52a
MW+WBF+FW+Bac	3.72±1.57abcd	0.18±0.07abc	0.23±0.15a	5.54±8.62a	860.89±1306.69a
MW+WBF+FW+Pse	3.60±1.60abcd	0.23±0.05a	0.23±0.15a	6.32±9.44a	904.67±1336.22a
MW+WBF+FW+Bac+Pse	3.74±2.10abcde	0.23±0.03a	0.24±0.13a	6.06±8.27a	1014.89±1523.37a

Key: UW - Uncontaminated Water, **MW** - Marine Water, **WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051, ***Pse*** - *Pseudomonas aeruginosa EB38*, **COD** - Chemical oxygen demand, **THC** - Total hydrocarbon content.

Table 6. Biodegradation of total hydrogen content (THC) in the treatments.

Treatment Code		Day1	Day14	Day28	Amount remediated	% Bioremediation
T1	UW	252.7±1.16	82.7±1.15	38.0±0.00	214.67	-
T2	MW+WBF	3282.7±1.16	1384.0±0.00	1234.0±0.00	2048.67	62.41
T3	MW+WBF+Bac	3282.7±1.17	128.0±0.00	46.0±0.00	3236.67	98.6
T4	MW+WBF+Pse	3282.7±1.18	174.0±0.00	88.0±0.00	3194.67	97.32
T5	MW+WBF+FW	3282.7±1.19	188.0±0.00	159.3±1.15	3123.34	95.15
T6	MW+WBF+FW+Bac	3282.7±1.20	156.0±0.00	58.0±0.00	3224.67	98.23
T7	MW+WBF+FW+Pse	3282.7±1.21	158.0±0.00	108.7±1.15	3174	96.69
T8	MW+WBF+FW+Bac+Pse	3282.7±1.22	175.3±2.31	96.0±0.00	3186.67	97.08

Key: UW - Uncontaminated Water, **MW** - Marine Water, **WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051, ***Pse*** - *Pseudomonas aeruginosa EB38*, **COD** - Chemical oxygen demand, **THC** - Total hydrocarbon content.

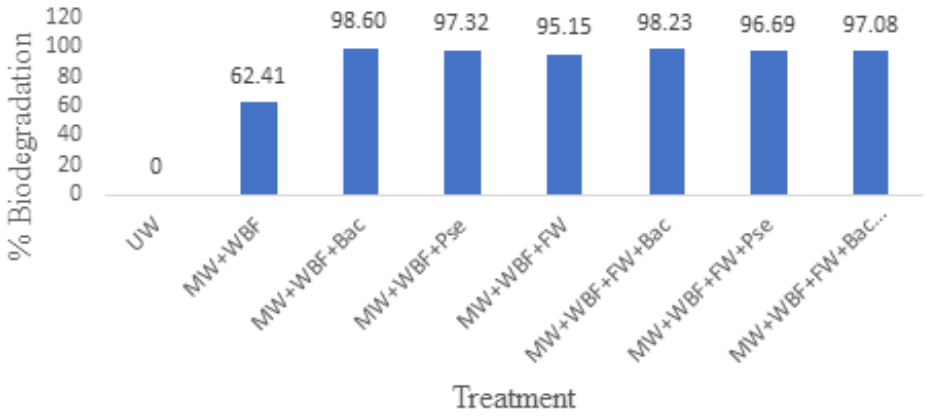

Figure 1. Percentage (%) total hydrocarbon content (THC) reduction during enhanced biodegradation of drilling fluids in marine water.

Key: UW - Uncontaminated Water, **MW** - Marine Water**, WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051, ***Pse*** - *Pseudomonas aeruginosa EB38.*

microbial inoculants (*Bacillus safensis* and *Pseudomonas aeruginosa*) and fish waste (FW) significantly enhanced the biodegradation of THC in drilling fluids. The highest bioremediation efficiency (98.6%) was achieved in Treatment T3 (MW+WBF+Bac), indicating that the addition of *Bacillus safensis* alone was highly effective in degrading THC. The combination of microbial inoculants and fish waste (Treatments T5-T8) also showed high bioremediation efficiencies (95.15-98.23%), suggesting a synergistic effect between the microorganisms and fish waste. The control treatment (T1, UW) showed minimal biodegradation, highlighting the need for additional treatments to enhance THC degradation.

Microbial population in the biodegradation setups

The result (Table 7) shows the mean and standard deviation of microbiological counts (THB, TFC, DFUF, and DFUB) during the enhanced biodegradation of drilling fluids in marine water. The treatments with the highest THB (Total Heterotrophic Bacteria) counts were MW+WBF+FW+Bac+Pse (18.87 cfu/ml) and MW+WBF+ Pse (17.59 cfu/ml), indicating a significant increase in bacterial growth. The TFC (Total Fungal Counts) remained relatively consistent across treatments, ranging from 3.11 to 5.11 cfu/ml. The DFUF (Drilling Fluid Utilizing Fungi) counts were highest in MW+WBF+FW+Bac+Pse (1.78 cfu/ml) and MW+WBF+FW+Pse (1.78 cfu/ml), suggesting enhanced fungal growth. The DFUB (Drilling Fluid Utilizing Bacteria) counts were highest in MW+WBF+FW+Bac+ Pse (12.76 cfu/ml) and MW+WBF+FW+Bac (13.20 cfu/ml), indicating increased bacterial utilization of drilling fluids.

The result (Figure 2) suggests that the addition of microbial inoculants (*Bacillus safensis* and *Pseudomonas aeruginosa*) and fish waste (FW) significantly enhanced the growth of microorganisms, leading to increased biodegradation of drilling fluids.

Table 7. Mean and standard deviation of microbiological counts during enhanced biodegradation of drilling fluids in marine water.

Treatment code	THB (cfu/ml)	TFC (cfu/ml)	DFUF (cfu/ml)	DFUB (cfu/ml)
UM	3.11±1.95efg	3.78±0.84a	3.00±3.76a	2.34±1.69def
MW+WBF	1.68±0.63fg	4.67±1.20a	2.56±1.50a	0.60±0.72f
MW+WBF+Bac	8.46±2.15cdefg	5.11±3.17a	2.00±0.88a	4.09±1.56cdef
MW+WBF+Pse	17.59±2.70ab	5.11±3.50a	2.44±1.26a	10.81±6.38abcde
MW+WBF+FW	14.74±4.09abcd	4.44±2.83a	3.22±0.19a	8.51±3.65abcdef
MW+WBF+FW+Bac	14.40±4.13abcd	4.44±1.17a	2.78±0.51a	13.20±3.70ab
MW+WBF+FW+Pse	15.77±1.52aabc	4.11±2.27a	1.78±1.35a	14.63±2.87a
MW+WBF+FW+Bac+Pse	18.87±6.10a	3.11±1.35a	1.78±0.38a	12.76±0.96abc

KEY: UW - Uncontaminated Water, **MW** - Marine Water**, WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051, ***Pse*** - *Pseudomonas aeruginosa EB38.*

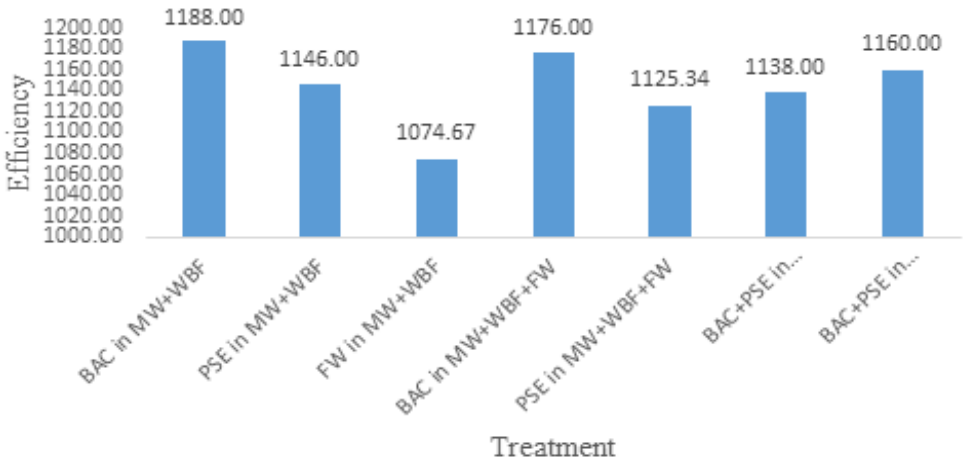

Figure 2. Efficiency of the different bioaugmenting organisms (*Pseudomonas* and *Bacillus*) and biostimulating agent (fish waste).

The increase in THB (Total Heterotrophic Bacteria) counts in treatments with microbial inoculants and fish waste indicates a boost in bacterial growth, which is essential for biodegradation. The consistent TFC (Total Fungal Counts) across treatments suggests that fungal growth was not significantly impacted by the additions. The increase in DFUF (Drilling Fluid Utilizing Fungi) and DFUB (Drilling Fluid Utilizing Bacteria) counts in treatments with microbial inoculants and fish waste indicates enhanced utilization of drilling fluids by microorganisms, leading to improved biodegradation. The highest microbial counts were observed in treatments with the combination of microbial inoculants and fish waste, suggesting a synergistic effect between the two.

The highest % efficiency of biodegradation in the experimental set-up was observed in the treatment MW+WBF+Bac, while the lowest percentage of 31.83% was observed in MW+WBF+FW+Bac+Pse, as shown in Figure 3.

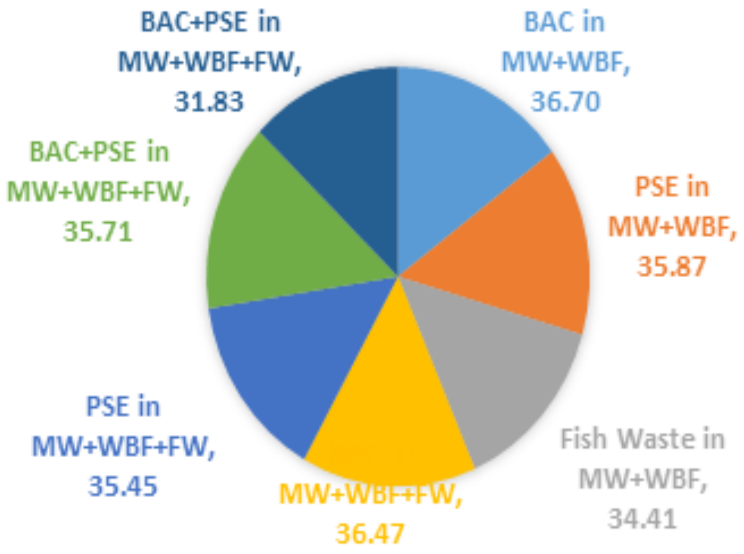

Figure 3. Percentage (%) efficiency of the bioaugmenting organisms (*Pseudomonas* and *Bacillus*) and biostimulating agent (fish waste).

KEY: UW - Uncontaminated Water, **MW** - Marine Water, **WBF** - Water Base Drilling Fluid, **FW** - Fish Waste, **Bac** - *Bacillus safensis strain* UIS0051 ***Pse*** - *Pseudomonas aeruginosa EB38.*

DISCUSSION

The marine water sample exhibited a neutral pH of 7.6, which is within the optimal range for microbial growth (Atlas and Bartha, 1998). The elevated temperature of 30°C was conducive to microbial growth, consistent with Madigan et al. (2017). However, the high levels of total dissolved solids (59330 mg/L) and electrical conductivity (12300 µS/cm) may potentially impact microbial activity, deviating from the optimal conditions reported by Clesceri et al. (1998). The low total suspended solids (0.01 mg/L) and undetectable levels of chlorine and bromine (<0.01 mg/L) indicated minimal particulate matter and disinfectant presence, aligning with Prince et al. (2010). The high alkalinity (3322.48 µeq/L) and hardness (4033.43 mg/L) may influence microbial growth and enzyme activity (Sterner and Elser, 2002). The low salinity (7.5 mg/L) and turbidity (0.013 NTU) suggested a relatively clear water sample, contrasting with the higher levels reported in marine environments by Head et al. (2006). The low dissolved oxygen level (2 mg/L) may limit aerobic microbial growth (Madigan *et al.,* 2017). The biochemical oxygen demand (1.94 mg/L) and chemical oxygen demand (3.41 mg/L) were low, indicating minimal organic pollution (Prince *et al.,* 2010). The nutrient levels, including nitrate (1.982 mg/L), sulphate (0.929 mg/L), and phosphate (0.231 mg/L), were low, potentially limiting microbial growth, consistent with Sterner and Elser (2002). However, the high total hydrocarbon content (220 mg/L) may support the growth of hydrocarbon-degrading microorganisms (Head et al., 2006). The marine water sample exhibited a robust microbial population, indicative of a high potential for biodegradation of drilling fluids (Madigan *et al.,* 2017). The total heterotrophic bacteria count of 1.45 ± 0.06 x 10^9 CFU/mL agrees with Atlas and Bartha (1998), who reported substantial biodegradation capacity at similar bacterial densities. The presence of drilling fluids utilizing bacteria (1.07 ± 0.08 x 10^5 CFU/mL) and fungi (5.67 ± 0.58 x 10^3 CFU/mL) further substantiates the water sample's biodegradation potential. These microorganisms can break down and utilize drilling fluids, contributing to environmental remediation efforts, as reported by Prince et al. (2010). The total fungi count of 1.45 ± 0.47 x 10^5 CFU/mL is noteworthy, as fungi play a crucial role in decomposing organic matter (Sterner and Elser, 2002). The coexistence of bacteria and fungi in the water sample suggests a synergistic relationship, enhancing biodegradation capabilities, similar to Clesceri *et al.* (1998). The microbial population's composition and density indicate a high potential for biodegradation of drilling fluids, making this marine water sample a suitable candidate for bioremediation efforts. The fish waste sample exhibited a slightly elevated temperature (31.83°C) compared to the marine water sample, suggesting potential microbial activity, consistent with Madigan et al. (2017). The pH of 6.28, slightly acidic, may favour the growth of specific microorganisms (Atlas and Bartha, 1998). The high electrical conductivity (13150 µS/cm) and total dissolved solids (65780 mg/L) indicate a highly concentrated solution, potentially impacting microbial growth and enzyme activity, consistent with Clesceri et al. (1998). Moderate levels of organic pollution were observed, with a biochemical oxygen demand (BOD) of 4.6 mg/L and chemical oxygen demand (COD) of 8.11 mg/L, indicating potential biodegradation capabilities, similar to Prince et al. (2010). Nutrient levels were relatively low, with nitrate (12.3 mg/L), phosphate (0.38 mg/L), and sulphate (0.05 mg/L) present in limited quantities, potentially limiting microbial growth, consistent with Sterner and Elser (2002). However, high levels of total hydrocarbon content (192.67 mg/L) were detected, posing potential environmental concerns due to toxicity and persistence, agreeing with Head et al. (2006).

Microbial analysis revealed moderate total heterotrophic bacteria (THB) counts (1.21±6.56 cfu/mL) and relatively high total fungi counts (TFC) (6.67±1.53 cfu/mL), indicating potential biodegradation capabilities (Madigan et al., 2017). The fish waste sample's microbial population and physicochemical parameters suggest a moderate potential for biodegradation, with possible environmental concerns due to high hydrocarbon levels.

The marine water isolates, both pre- and post-contamination with drilling fluids, exhibited a diverse array of biochemical and morphological characteristics, agreeing with Madigan *et al.* (2017). The presence of bacterial species such as *Pseudomonas* sp., *Bacillus* sp., *Proteus* sp., and *Micrococcus* sp. is consistent with Atlas and Bartha (1998), who reported these genera as common in marine environments. The fungal genera, including *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Candida* sp., and *Saccharomyces* sp., align with Sterner and Elser (2002), who implicated these genera in decomposition and biodegradation processes. The diverse microbial population in the marine water sample suggests a high potential for biodegradation of drilling fluids, as reported by Head et al. (2006). The presence of these microorganisms before and after contamination indicates their adaptability and ability to thrive in environments with high levels of drilling fluids (Prince et al., 2010). Overall, the microbial diversity in the marine water sample showed its potential for bioremediation and environmental remediation efforts, highlighting the importance of harnessing Indigenous microorganisms for pollution mitigation.

During biodegradation monitoring, significant changes in physicochemical characteristics were observed. Temperature ranged from 29.80°C to 30.37°C, indicating minimal thermal impact, similar to Madigan et al. (2017). pH levels ranged from 6.92 to 7.20, suggesting a slightly acidic to neutral environment, aligning with Atlas and Bartha (1998). Total Dissolved Solids (TDS) increased significantly, from 26465 mg/L to 65577 mg/L, indicating a substantial increase in dissolved substances. Electrical

Conductivity (EC) values ranged from 12590 µS/cm to 48950 µS/cm, suggesting a significant increase in ionic strength, similar to Clesceri et al. (1998). Dissolved Oxygen (DO) levels ranged from 2.08 mg/L to 5.32 mg/L, indicating a moderate increase in oxygen availability. Biological Oxygen Demand (BOD) values increased from 0.76 mg/L to 3.44 mg/L, suggesting a significant increase in organic pollution. Nutrient levels and organic pollution indicators varied across treatments, with nitrate levels increasing significantly, from 0.46 mg/L to 3.89 mg/L, indicating potential nitrogen enrichment. Phosphate levels showed a moderate increase, from 0.01 mg/L to 0.23 mg/L, suggesting potential phosphorus enrichment. Sulphate levels remained relatively consistent, ranging from 0.23 to 0.27 mg/L, indicating minimal changes in sulphur compounds. Chemical Oxygen Demand (COD) levels increased significantly, from 1.22 mg/L to 6.32 mg/L, indicating a substantial increase in organic pollution. Total Hydrocarbon Content (THC) levels showed a dramatic increase, from 124.44 mg/L to 1966.89 mg/L, indicating significant hydrocarbon contamination. Biodegradation of THC was observed in all treatments over 28 days, with the highest percentage of bioremediation achieved in Treatment T3 (MW+WBF+Bac) at 98.6%, suggesting that *Bacillus safensis* significantly enhanced THC biodegradation.

CONCLUSION

The marine water sample exhibited a diverse range of microorganisms with a high potential for biodegradation of drilling fluids. The presence of bacterial species such as *Pseudomonas* sp, *Bacillus* sp, *Proteus* sp, and *Micrococcus* sp, as well as fungal genera including *Aspergillus* sp, *Penicillium* sp, *Mucor* sp, *Rhizopus* sp, *Candida* sp, and *Saccharomyces* sp, indicates a high potential for bioremediation and environmental remediation efforts. The addition of microbial inoculants (*Bacillus safensis* and *Pseudomonas aeruginosa*) and fish waste (FW) significantly enhanced the biodegradation of Total Hydrocarbon Content (THC), with the highest percentage of bioremediation achieved in Treatment T3 (MW+WBF+Bac) at 98.6%. The results suggest that microbial inoculants and fish waste can be effective amendments for enhancing the biodegradation of THC in drilling fluids, with potential applications in environmental remediation.

RECOMMENDATION

The use of microbial inoculants, such as *Bacillus safensis* and *Pseudomonas aeruginosa*, and fish waste should be considered to enhance the biodegradation of Total Hydrocarbon Content in drilling fluids. Further research is needed to optimize the conditions for biodegradation, including temperature, pH, and nutrient levels. Bioremediation efforts should be monitored to assess their effectiveness in mitigating the impacts of drilling fluids on marine ecosystems. The potential for fish waste to serve as a nutrient source for microbial growth should be further investigated. Additional microbial species should be explored for their potential to contribute to the biodegradation of drilling fluids. A comprehensive remediation strategy should be developed and implemented, incorporating bioremediation alongside other methods to ensure environmental protection.

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