Available online at http://www.journalijdr.com

IJDR

ISSN: 2230-9926

International Journal of Development Research
Vol. 07, Issue, 12, pp.17872-17876, December, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

EFFECT OF WASTEWATER ON BIODEGRADABILITY OF DRILLING FLUID

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ARTICLE INFO

Article History:

Received 10th September, 2017
Received in revised form
19th October, 2017
Accepted 14th November, 2017
Published online 30th December, 2017

Key Words:

Biodegradation,
Oil and water base drilling fluid,
Waste water,
Hydrocarbon Utilizing Bacteria and Fungi.

ABSTRACT

This study reports the effect of waste water on biodegradation of drilling fluid contamination in brackish water environment. The experimental set ups include; Control 1(Brackish water with Water base drilling fluid) (BW+WB), Control 2 (Brackish water with Oil base drilling fluid) (BW+OB), Brackish water with Water base drilling fluid with Waste water (BW+WB+WW) and Brackish water with Oil base drilling fluid with Waste water (BW+OB+WW). Biodegradability was monitored for 28days. The average microbial load of the biodegradation monitoring for days 1, 7, 14, 21, and 28 were; Total Heterotrophic Bacterial (log_{10}cfu/ml): Control 1 (BW+WB) 8.89±0.48, Control 2(BW+OB) 7.89±2.27, BW+WB+WW 8.85±0.82, BW+OB+WW 6.54±1.59; Hydrocarbon Utilizing Bacterial (log_{10}cfu/ml): Control 1 (4.24±0.67), Control 2 (BW+OB) 4.33±0.56, BW+WB+WW 4.65±0.66, BW+OB+WW 4.92±0.77; Hydrocarbon Utilizing Fungi (log_{10}cfu/ml): Control 1 (BW+WB) 4.23±0.61, Control 2 (BW+OB) 7.13±2.95, BW+WB+WW 8.01±2.61, BW+OB+WW 5.95±1.82. Major genera of drilling fluid utilizing bacteria and fungi isolated were; *Bacillus*, *Pseudomonas*, *Micrococcus, Proteus*; *Mucor, Aspergillus, Rhizopus* and *Penicillium.* The percentage (%) residual hydrocarbons at day 28 were; BW+WB+WW 0.65 < BW+WB 1.59 < BW+OB+WW 6.86 <BW+OB 15.41 while the cumulative percentage (∑%) effect of waste water on the drilling fluids revealed; Waste water on Oil base 44.52 greater than Waste water on Water base 40.88. The percentage effect of waste water per mg/kg of hydrocarbon degradable in Oil base drilling fluid is greater than Water base drilling fluid by 3.64∑%. Note: 1.0∑% = 921.44mg/kg Total Hydrocarbon. The success of the test could be due to the high microbial load found in the waste water especially hydrocarbon utilizing micro flora; thus could be used as enhancer in biodegradation of hydrocarbon contamination environment. However pathogenic waste water microbes should be screened out before use.

Citation: Renner R. Nrior, Salome Ibietela Douglas and Martha Tamunoemi Ojongo, 2017. "Effect of wastewater on biodegradability of drilling fluid", *International Journal of Development Research*, 7, (12), 17872-17876.

INTRODUCTION

The utilization of petroleum hydrocarbon by bacteria is one of the primary ways of eliminating petroleum hydrocarbon from contaminated soil and appears to be the most environmentally friendly method (Korda *et al.,* 2000). One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Nrior and Echezolom, 2016). Currently accepted disposal methods of incineration or burial insecure landfills can become prohibitively expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive (Hii, 2007). Biodegradation/Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants

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(Rocchetii *et al.*, 2012; Nrior and Echezolom, 2016). Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants (Nrior and Mene, 2017). Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy (Trejo *et al.*, 2007; Nrior *et al.*, 2017a,b). Waste water, is any water that has been adversely affected in quality by anthropogenic influence. Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration. Municipal wastewater (also called sewage) is usually conveyed in a combined sewer or sanitary sewer, and treated at a wastewater treatment plant (Chaillian *et al*, 2004). The biodegradability of petroleum products is dependent on the chemical structure of their various components. Compound resistance to biodegradation increases with increasing molecular weight. However, the relations between hydrocarbon physico-chemical properties and biodegradability have been little studied. Several works (Nrior and Odokuma, 2015), dealing with laboratory techniques of biodegradability determination and the influence of experimental conditions, showed the variation of the results according to the used method and considered conditions (Pinzon-martinez, *et al.*, 2010). In general, the more soluble, lighter petroleum hydrocarbons are more biodegradable than the less soluble, heavier members of the group. Viscosity is also known to have an important impact on biodegradability. Highly viscous hydrocarbons are less biodegraded because of the inherent physical difficulty in establishing contact among contamination and microorganisms, nutrients, and electron acceptors compounds (Ojo, 2005). The viscous diesel oil at high amount (>10%) shows low biodegradation rate (4%), but, in the presence of mixed culture (*Enterobacter, Citrobacter, Pseudomonas, Staphylococcus, Bacillus, Micrococcus*) it presents good biodegradation properties (Khodja, 2008). Biodegradability is an important characteristic of drilling fluids to ensure that the fluids do not persist in the environment for long periods of time. Biodegradation rates are influenced by factors like seabed temperature, fluid concentrations and loadings, the surface area of the cuttings pile, and sediment particle size (Elshajie *et al*., 2007). There are essentially three main categories of drilling fluids: oil based fluids (OBF's), synthetic based fluids (SBF's) and water based fluids (WBF's). Oil based fluids have traditionally been used for their high performance drilling characteristics but tend to have a poor environmental friendliness in terms of their ecotoxicity and their tendency to persist in cuttings piles on the seafloor (Korda *et al.,* 2000; Nrior *et al*., 2017a). Therefore, this work is aimed at evaluating the effect waste water on the biodegradability of oil and water based drilling fluid in freshwater environment.

MATERIALS AND METHODS

Collection of Samples

Drilling fluids – water and oil base was gotten from Addax Petroleum Limited, Izombe, Owerri, Imo State Nigeria. Waste water sample was gotten from Rumuokoku Street, Port Harcourt, Nigeria. Brackish water samples were collected with sterile plastic ten (10) litre containers from Eagle Island in Port Harcourt 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.

Biodegradation Test

Biodegradation setup were carried out in four set, each setup in 500ml conical flask containing 4ml of each drilling fluid (oil base or water base) with 396ml of the brackish water. The control flask contained 4ml of drilling fluid, 396ml brackish water and 20ml of wastewater samples (Table 1 *Biodegradation Experimental Set up*). The biodegradation of each test sample was determined by conducting microbiological (Total heterotrophic Bacteria, Total fungi, and Hydrocarbon Utilizing Bacteria and fungi) and physicochemical analysis of the samples collected from biodegradation flask at days 1, 7, 14, 21 and 28.

Media Used

Nutrient Agar: Nutrient Agar of L:S – Brotech company was used for the isolation and enumeration of total heterotrophic bacteria. The media was suspended by weighing 28gm into 1000ml of distilled water according to the manufacturer's specification. It was mixed thoroughly and autoclaved at 15 psi, 121^0C for 15minutes and was aseptically poured into Petri-dishes.

Sabouroud Dextrose Agar: Sabouroud dextrose agar of Titan Biotech limited was used for the isolation of total fungal. 65 grams of the medium was suspended in 1000ml of distilled water according to the manufacturer's specification. It was mixed thoroughly and autoclaved at 15psi, 121^0C for 15minutes and was aseptically poured into Petri dishes.

Oil Agar Medium: Oil agar medium was prepared according to the modified mineral salts medium (MSM) composition of Mills *et al*., (1978). The composition of this media is as follows: K_2 $HPO_4.7H_2O$ (0.5g), $MgSO_4$ (0.3g), $NaCl_2$ (0.3g), $MnSO_4$ H_2O (0.2g), $FeSO_4.H_2O$ (0.02g), $NaNO_3$ (0.03g), $ZnCl_2$ (0.03g), Agar Agar (16g), Distilled water (200ml). The media was prepared by adding 1% of crude oil to this mineral salt medium. The medium was used for the isolation of total petroleum degrading bacteria. The medium was mixed thoroughly and autoclaved at 15psi at 121^0C for 15 minutes and it was allowed to cool to 45^0C and was aseptically poured into Petri dishes.

Characteristics and Identification of Isolates

Identification of bacterial isolates was based on their cultural morphology, microscopic examination, carbohydrate fermentation and other biochemical test. References were made to Bergey's Manual of Determinative Bacteriology (1974), 8th Edition for the identification of bacteria. Macroscopic examination was done by physical characteristics of the mycelia for colour and structure. Microscopic characteristics were also done through the morphological structure; wet mount method was done before viewing the isolates under ×40 objective of the microscope. The morphological structures include septate, non-septate, presence of sporangiophores, fruiting bodies and special organism like rhizoids etc.REF

Table 1. Biodegradation Experimental Set up.

Set up labels	Habitat water	Drilling Fluid		Waste water	Final volume
		Water base	*Oil base*		
Control 1 (BW+WB)	396ml	4ml (1%)	-	-	400ml
Control 2 (BW+OB)	396ml	-	4ml (1%)	-	400ml
BW+WB+WW	376ml	4ml (1%)	-	20ml	400ml
BW+OB+WW	376ml	-	4ml (1%)	20ml	400ml

Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water.

Biodegradation of drilling fluid monitoring

Biodegradation of drilling fluid monitoring Total hydrocarbon content (THC) analysis: Total Hydrocarbon Content (THC) analyses were carried out on all the six setups using spectrophotometric method for Days 1, 7, 14, 21 and 28. (Nrior *et al.*, 2017b)

Percentage (%) biodegradation evaluation

The percentage (%) biodegradation is 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 final concentration of pollutant at end of 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;

$B_C = I_C - F_C$

$Bx = Ic - I_0$

Where,

B_C=Amount of pollutant degraded

I_C=Initial concentration of pollutant (Day 0)

F_C=Final concentration of pollutant at end of experiment (Last day)

I_0=Initial concentration value of Control at day 0

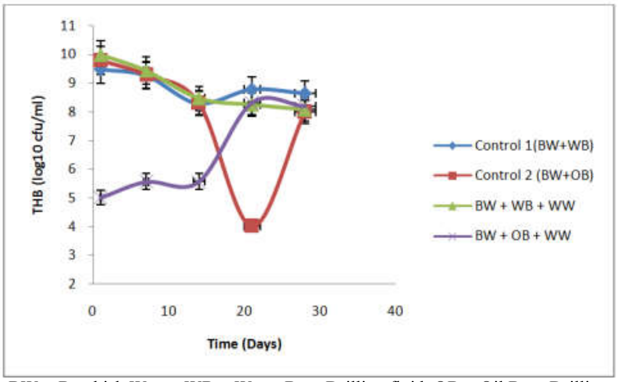
B_x=Actual amount of pollutant in test medium

$$\% \text{ Biodegradation} = \frac{B_C \times 100}{Bx}$$

RESULTS AND DISCUSSION

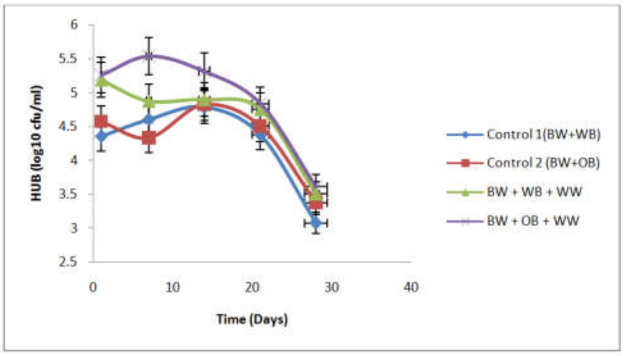
Microbiological evaluation during biodegradation of drilling fluid (oil base and water base)mixed with waste water in brackish ecosystem revealed Hydrocarbon Utilizing Bacterial isolates obtained from different mixture of drilling fluid with waste water belong to the genera; *Pseudomonas*, *Bacillus*, *Proteus*, *Micrococcus* and fungi; *Aspergillus, Penicillium, Mucor, Rhizopus* and yeast such as *Candida* and *Saccharomyces*. Total heterotrophic bacteria (THB) count decreases from day 1 to day 28; with Control 1 (BW+WB) having the highest cumulative average count (8.89±0.48 $\log_{10}$cfu/ml) (Figure 1 *Total Heterotrophic Bacteria (THB) ($\log_{10}$cfu/ml) during biodegradation of drilling fluid*). Statistical evaluation of THB growth data during the biodegradation of drilling fluid with waste water revealed the following order: Total Heterotrophic Bacteria (THB – $\log_{10}$cfu/ml): Control 1 (BW+WB) 8.89±0.48>BW+WB+WW 8.85±0.82> Control 2(BW+OB) 7.89±2.27>BW+OB+WW 6.54±1.59. Generally, this meant that the intermediates produced from the degradation of water base and oil base drilling fluid mixed with waste water in brackish ecosystem favoured the growth of a larger population of heterotrophic bacteria. Six genera of Hydrocarbon Utilizing Fungi (HUF) identified were – *Aspergillus*, *Penicilium*, *Rhizopus, Mucor, Candida* and *Saccharomyces*. The trend observed in HUB was same with total fungal population. Statistical evaluation of the growth of total fungi (Figure 2 *Hydrocarbon Utilizing Bacteria (HUB) (log10 cfu/ml) during biodegradation of drilling fluid with waste water*) during the biodegradation of water base and oil base drilling fluid mixed with waste water in brackish water ecosystem revealed the following Hydrocarbon Utilizing Fungi (HUF - log10 cfu/ml): BW+WB+WW 8.01±2.61 > Control 2 (BW+OB) 7.13±2.95 >BW+OB+WW 5.95±1.82>Control 1 (BW+WB) 4.23±0.61. These test water base and oil base drilling fluid mixed with waste water in brackish water ecosystem showed mild increases and decreases in the HUF population. This observation is in agreement with the report of Okpokwasili and Nnubia (1995); Nrior and Wosa (2016); Nrior *et al.* (2017a) 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. Responding to changes in the environment is a fundamental property of a living cell and chemotaxis is the best studied bacterial behavioural response that navigates the bacteria to niches that are optimum for their growth and survival (Bren and Eisenbach, 2000). Acclimatization of the microbial population with drilling fluids components enhances the biodegradation efficiency of the microorganisms.

Although bacterial population was more than fungal drilling fluids utilizers in the waste water, this agrees with previous findings of Okpokwasili and Olisa (1991), Amund *et al*., (1997), Nrior and Wosa (2016), Nrior *et al*., (2017b). The adaptability of native microbial population in the waste water brackish water systems to drilling fluids components would be the reason for their success at mineralizing the water base and oil base drilling fluids in the experimental set-up where the physico-chemical properties of the ecosystem were supportive to the survival of these microorganisms (Spain and van Veld, 1983; Nrior *et al*., 2017b). Evaluation of Hydrocarbon utilizing bacteria (HUB)during the biodegradation of water base and oil base drilling fluid mixed with waste water in brackish water ecosystem (Figure 3 *Hydrocarbon Utilizing Fungi (HUF) ($\log_{10}$cfu/ml) during biodegradation of drilling fluid with waste water*) revealed their population in the following order; Hydrocarbon Utilizing Bacteria (HUB-$\log_{10}$cfu/ml): BW+ OB+WW 4.92±0.77>BW+WB+WW 4.65±0.66>Control 2 (BW+OB) 4.33±0.56> Control 1 (4.24±0.67). The study revealed bacterial genera; *Pseudomonas*, *Bacillus*, *Proteus*, *Micrococcus* and fungi; *Aspergillus, Penicillium, Mucor, Rhizopus* and yeast such as *Candida* and *Saccharomyces* isolated from the waste water with brackish water mixture were capable of utilizing drilling fluid as their carbon source. Similar trend in the ability of natural microbiota to degrade novel or synthetic compounds has been reported. 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; Nrior and Wosa, 2016).



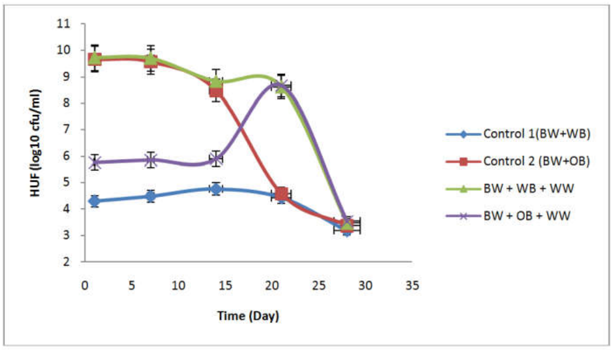
Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water

Fig.1. Total Heterotrophic Bacteria (THB) (log_{10}cfu/ml) during biodegradation of drilling fluid with waste water in brackish water ecosystem



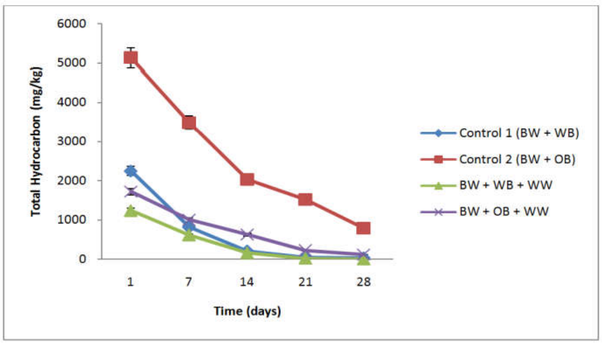
Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water.

Fig.2. Hydrocarbon Utilizing Bacteria (HUB) (log10 cfu/ml) during biodegradation of drilling fluid with waste water in brackish water ecosystem



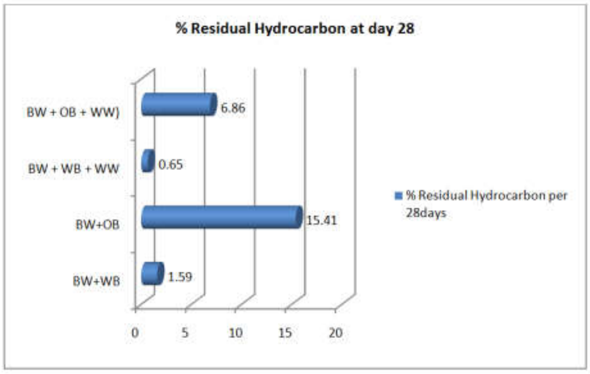
Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water.

Fig.3. Hydrocarbon Utilizing Fungi (HUF) (log_{10}cfu/ml) during biodegradation of drilling fluid with waste water in brackish water ecosystem



Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water.

Fig.4. Total Hydrocarbon Content (THC) (mg/kg) during biodegradation of drilling fluid with waste water in brackish water ecosystem



Key: BW = Brackish Water, WB = Water Base Drilling fluid, OB = Oil Base Drilling Fluid, WW = Waste Water.

Fig.5. Percentage (%) residual hydrocarbon at day 28 during biodegradation of drilling fluid with waste water in brackish water ecosystem

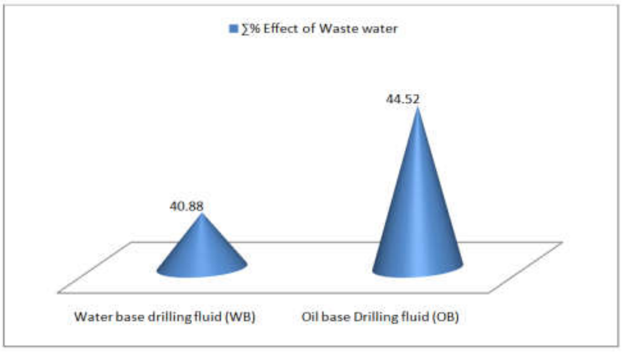


Fig. 6. Cummulative percentage (∑%) effect of Waste water on biodradation of drilling fluids (water base and oil base) in brackish water ecosystem

Hence, the ability to utilize xenobiotics must be dependent on the possession of the requisite enzymes necessary for such degradation (Nrior and Odokuma, 2015). Total Hydrocarbon Content (THC) (mg/kg) during biodegradation of drilling fluid with waste water in brackish water ecosystem revealed a sharp and continuous decrease in the concentration of the sample from Day 7 until the end of the experiment (Figure 4 *Total Hydrocarbon Content (THC) (mg/kg) during biodegradation of drilling fluid with waste water*). The decrease observed in the test samples is a clear indication that the drilling fluids were mineralized by the bacterial isolates and some fungal isolates, while that observed in the control sample may be as a result of decrease in nutrient concentration of the aquatic system. The percentage (%) residual hydrocarbons at the day 28 were; BW+WB+WW 0.65< BW+WB 1.59 < BW+OB+WW 6.86 < BW+OB 15.41 (Fig. 5. *Percentage (%) residual hydrocarbon at day 28 during biodegradation of drilling fluid with waste water*). The cumulative percentage (∑%) effect of waste water on the drilling fluids revealed; Waste water on Oil base drilling fluid in brackish water (OB+BW+WW) 44.52 which is greater than Waste water on Water base drilling fluid in brackish water (WB+BW+WW) 40.88. The percentage effect of waste water per mg/kg of hydrocarbon degradable in Oil base drilling fluid is greater than Water base drilling fluid by 3.64 ∑%. Note: 1.0∑% = 921.44mg/kg Total Hydrocarbon (Fig. 6. *Cummulative percentage (∑%) effect of Waste water on biodradation of drilling fluids*). This could probably due to the high content of hydrocarbon in milligram per Litre of oil base drilling fluid than that of water base drilling fluid. The results obtained however, reveals the ability of waste water to enhance the degradation of the two types of drilling fluids (water base and oil base) evaluated.

Conclusion

The most effective method for the elimination of contaminants may be achieved by using waste water with high hydrocarbon (or other pollutants) utilizing microorganisms to help degrade the pollutant/contaminant. The success of this biodegradation strongly depends on the survival of the microorganism thereby ensuring adequate temperature, pH, salinity, nutrients and oxygen content, because the activities of these microorganisms are very important for the renewal of our environment and maintenance of our environment. Cleaning up hydrocarbons (drilling fluid/ drilling mud) after crude oil drilling operations or related pollutants in our environment is a real world problem. A better understanding of the mechanism of biodegradation has high ecological significance that depends on the action of indigenous microorganisms to transform or mineralize the pollutants. Therefore, based on the present research, it may be concluded that microbial degradation using screened waste water can be considered as a key option in the cleanup strategy for petroleum hydrocarbon remediation/ biodegradation.

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