

Validity of Bioremediation Studies Utilizing Engine Oil as the Spiking Fluid Intended for Formulations of Effective Bioremediation Approach for Crude Oil Impacted Soils

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Abstract:

There has been a controversy as to whether engine oil is a valid substitute to ascertain a remediation approach' ability to remediate crude oil impacted soils. So far, bioremediation research has been with either engine oil or crude oil as the spiking fluid, there is no record that both has been deployed in single research to evaluate the possible difference that may exist. Following that many published research on crude oil bioremediation made use of engine oil as the spill simulation fluid instead of crude oil and that this research originally planned for spill simulation using crude oil came close to adopting engine oil as the actual simulation fluid instead of the planned simulation fluid due to difficulties encountered in obtaining crude oil, a need arose to ascertain if engine oil is a true representative of crude oil in bioremediation research intended for formulations of effective bioremediation approaches to be replicated in the treatment of crude oil impacted soils. Two different set up categories containing the same quantity of same soil type were spiked separately with the same quantity (60ml) of crude oil and engine oil. Even though they yielded different values of Total Petroleum Hydrocarbon (TPH) which was expected considering the difference in colouration observed in the TPH extract of the two sets of samples during the TPH extraction process; both set ups experienced reduction in TPH levels following applied remediation approach. Also, desktop analysis of the chromatograms of both showed no significant difference. Thus, this research has shown or experimentally validated that in the absence of crude oil, engine oil can effectively be used for bioremediation research that can be replicated in the treatment of crude oil impacted soils.

Keywords —Bioremediation, Biostimulation, Phytoremediation.

1.INTRODUCTION

In reality, crude oil and engine oil are two different fluids; it is therefore understandable when one argues the use of one to determine the ability of a remediation approach in restoring soils impacted by the other. Crude oil is a mixture of compounds of hydrogen and carbon (hydrocarbons) and may include small amounts of non-hydrocarbons such as sulfur, nitrogen, and oxygen. Crude oil is refined to produce a wide array of petroleum products such as heating oils; gasoline, diesel and jet fuels; lubricants such as engine oil. Because engine oil is made from crude oil, it still contains hydrocarbons and may also contain traces of non-hydrocarbon compounds such as sulfur and nitrogen and mostly include added additives for performance improvement.

Despite the argument or controversy in the use of engine oil as the spiking fluid for bioremediation research intended for deployment in crude oil impacted soil treatment, various researchers as evident in the numerous published articles made use of engine oil as the spiking fluid. This could be attributed to the fact that engine oil is readily available to all while crude oil is available to a few. To cite but a few, Mohammad, et al (2022) investigated the role of organic wastes in stimulating the removal of petroleum hydrocarbon from contaminated soil but the spiking fluid used was spent engine oil; Ogbeh, (2019) carried out a study on nutrient optimization for bioremediation of spent engine oil contaminated soils, and the parameter that was used to check for biodegradation of the spent engine oil was Total Petroleum Hydrocarbon (TPH); Adenipekun, et al (2008) tested the efficacy of white rot fungus in degrading engine oil in soil and the concluded percentage of degradation was based on TPH parameter; Agarry, et al (2012) using biostimulation remediation method utilizing pig manure (organic nutrient), NPK (organic nutrient) and tween 80 (non-ionic surfactant) as biostimulants or biostimulating agents in soil spiked with engine oil analysed for TPH reduction as indices for the effectiveness of the applied approach and concluded that the applied biostimulation strategies can reduce the remediation

period of petroleum hydrocarbon contaminated environment; and Abioye, et al (2012) carried out a study aimed to determine the potential of certain remediation approaches for enhanced biodegradation of used lubricating oil in soil but TPH determination and reduction monitoring was used as the indices to conclude that there was rapid and high biodegradation of the used lubricating oil with 92% as the highest degradation percentage observed in the low contaminated soils and 55% as the highest degradation percentage observed in the high contaminated soils.

Nevertheless, the question remains, does the readily unavailability of crude oil make the readily available engine oil a perfect substitute for determining a remediation approach ability to remediate crude oil impacted soils? This is the question that the study sought to address. There is presently no record where engine oil alongside crude oil have been deployed in a single research in a bid to validate the use of engine oil in place of crude oil; this gap was recognized in this study and a hypothesis was therefore developed to bridge the gap.

2. MATERIALS AND METHODS

The study was carried out on a bench scale. 4kg of soil was measured and poured into plastic containers (already labelled for accurate identification). The set ups were categorized into two, while one was spiked with engine oil, the other was spiked with crude oil of the same quantity (60ml). The soil sample in each container was properly homogenized after the spiking process. About 20grams of soil was then taken from each of the plastic containers for analysis to ascertain initial values of Total Petroleum Hydrocarbon (TPH). Samples were also analysed during monitoring and at research close out.

Total Petroleum Hydrocarbon (TPH) Determination

Extraction of petroleum hydrocarbons was done using EPA 3550C (USEPA, 2007) standard operating procedure. The soil samples were thoroughly mixed in the sample plate and about 5

grams was removed from different points and placed inside the sample bottle. 2 spatula spoons of sodium sulphate were added to the 5 grams of soil in the sample bottle and properly homogenized before transferring into a conical flask. Sodium sulphate served moisture absorption purpose. 30ml of Dichloromethane and Acetone mixed in a ratio of 1:1 was added to the sample in the flask. The conical flasks were then covered with a foil and placed on a mechanical shaker and allowed to stay at 200 rotation speed for about 30 minutes. The agitation of the soil particles in the sample while on the electronic shaker assisted in extracting the petroleum hydrocarbons from the soil. Whatman filter paper was folded and placed on the aperture of the beaker, 4 to 5 grams of sodium sulphate was poured unto the filter paper and about 30 to 40 ml of Dichloromethane – Acetone mixture was introduced to wet the paper. The flask was removed from the mechanical shaker after 30 minutes and the liquid portion of the sample (the supernatant) was carefully poured into the beaker through the filter paper stuffed with sodium sulphate while ensuring that the residue (soil particles) does not go in as well. The liquid sample was allowed to properly filter through into the beaker before disposal of the filter paper along with its content. The beakers were then transferred to a water bath at about 65⁰C and allowed to stay uncovered until all the liquid evaporated leaving behind obtained petroleum hydrocarbon extract evidenced mainly by colouration at the base/lower section of the beaker. The extract was concentrated to 2ml by introducing 2ml of Dichloromethane – Acetone mixture into the beakers and they were properly shaken before pouring each into a separate graduated agilent vial with screw cap. To ensure the vial is free of water, it was dried with Dichloromethane – Acetone mixture before the sample was introduced into it.

GC method was used for data acquisition (from the petroleum hydrocarbon extract) with FID detector. Agilent technologies 6890N network GC system with auto-injector (agilent 7683 series injector model) was used. The analysis was run in splitless mode with agilent HB5 as the column of separation and an inlet temperature of 300⁰C at a pressure of 6psi. The oven program was set at an

initial temperature of 40⁰C to be held for 1 minute after which the temperature increases at 20⁰C per minute till it gets to 310⁰C and sustained for 15minutes. The detector temperature was set at 315⁰C with hydrogen (40.0ml/min) as the flow gas, helium (25.0ml/min) as the make-up flow, and air flow of 300ml/min.

Extracted TPH samples were loaded in the auto-sampler of the GC and the sample ID of samples in each tray position was inputted into the system before clicking on run sequence. The syringe volume of the auto-injector is 10 micro litter while the injection volume (that is, the volume of the petroleum hydrocarbon extract it will take from the graduated agilent vial loaded on the sampler) is 1 micro litre. The GC runs the analysis by introducing the sample extracts into the capillary column using the injection method. As the temperature of the column gradually increases, TPH compounds are separated according to their boiling points as they move towards the end of the column onto the flame ionization detector where the TPH compounds leaving the column are trapped and ionized by the hydrogen flow gas causing the gas in the detector to conduct electricity. Generated data (detector responses) are then displayed on the attached computer monitor. Detector responses in a given range are then integrated to give the total concentration of petroleum hydrocarbons present in the sample using Agilent Chemstation software (version B.04.03).

3. RESULTS AND DISCUSSION

During the laboratory TPH extraction process, by visual observation, it was obvious that less was extracted from the sample spiked with engine oil compared to the sample that is spiked with crude oil (see figure 1). It was therefore not surprising that after the GC-FID analysis of the extracted sample, the sample spiked with engine oil was found to have a lower average TPH value of 287 mg/kg compared to the sample spiked with crude oil that has average TPH value of 582mg/kg even though they were spiked with the same quantity and are of the same soil type. There was however, no

significant difference in their chromatograms except that C8 was not indicated in the chromatogram of the sample that was spiked with engine oil and that the integration process indicated reduced values of the remaining hydrocarbon components (C9 to C35) compared to that of the sample spiked with crude oil (see figure 2 and 3 as well as table 1 and 2), hence, the reduced TPH value. Howbeit, TPH reduction monitoring showed that the engine oil set ups experienced reduction in TPH values just like set ups spiked with crude oil following the applied bioremediation approach.

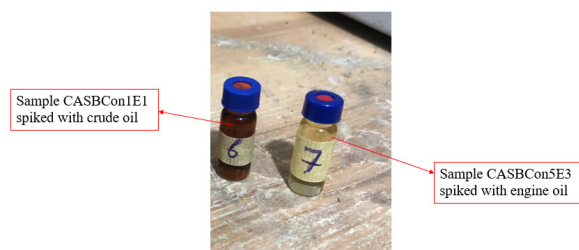


Fig. 1: TPH extract from soil sample spiked with crude oil and soil sample spiked with engine oil

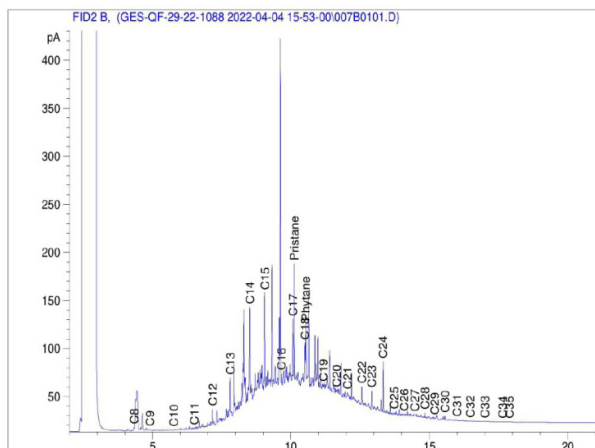


Fig. 2: TPH chromatogram of sample spiked with crude oil

Table 1: Quantitative report for TPH analysis for sample spiked with crude oil

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.514	VV	4.13750	3.37151	5.57984	C11	
7.179	VV	35.26451	7.02555e-1	9.91011	C12	
7.821	VV	84.88976	4.87912e-1	16.56751	C13	
8.520	VV	241.77499	3.78738e-1	36.62771	C14	
9.067	VV	244.42755	3.86360e-1	37.77483	C15	
9.661	VV	140.39290	4.47194e-1	25.11313	C16	
10.072	VV	314.56079	3.77538e-1	47.50343	C17	
10.127	VV	281.67761	3.86469e-1	43.54383	Pristane	
10.511	VV	206.50847	4.12698e-1	34.09024	C18	
10.537	VV	172.49710	4.54835e-1	31.38309	Phytane	
11.177	VV	57.91765	6.89073e-1	15.96381	C19	
11.655	VV	74.09049	6.21810e-1	18.42808	C20	
12.055	VV	215.27829	4.33545e-1	37.33315	C21	
12.569	VV	174.89012	4.62671e-1	32.36662	C22	
12.926	VV	80.83259	6.32522e-1	20.45137	C23	
13.338	VV	103.30173	5.99757e-1	24.78239	C24	
13.738	VV	66.51333	7.30830e-1	19.44398	C25	
14.100	VV	23.56117	1.40631	13.25375	C26	
14.473	VV	25.97243	1.33079	13.82556	C27	
14.825	VV	23.95629	1.39405	13.35849	C28	
15.188	VV	12.80702	2.20130	11.27683	C29	
15.552	VV	32.42075	1.15593	14.99043	C30	
15.982	VV	13.31751	1.99951	10.65141	C31	
16.468	VV	5.97614	3.35882	8.02912	C32	
16.999	VV	5.45824	3.17066	6.92249	C33	
17.635	VV	6.95495	2.29838	6.39406	C34	
17.894	VV	1.47451	20.61497	12.15881	C35	
19.012	-	-	-	-	C36	
19.932	-	-	-	-	C37	
21.016	-	-	-	-	C38	
22.443	-	-	-	-	C39	
23.834	-	-	-	-	C40	
Totals :				582.34663		

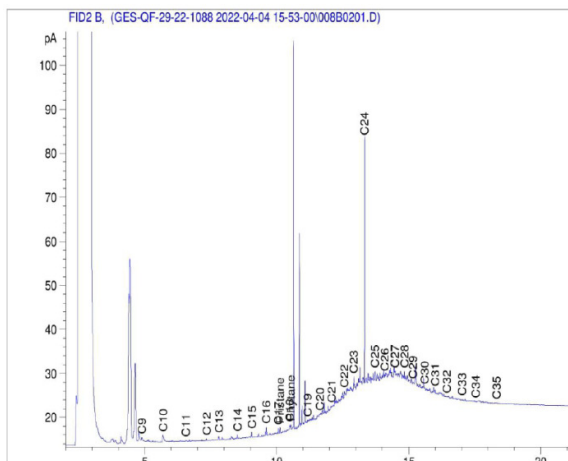


Fig. 3: TPH chromatogram of sample spiked with engine oil

Table 2: Quantitative report for TPH analysis for sample spiked with engineoil

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.565	VV	6.30018e-1	20.45278	5.15425	C11	
7.347	VV	5.42309e-1	26.07589	5.65648	C12	
7.816	VB	1.46603	10.67396	6.25936	C13	
8.521	VV	1.44992	11.64110	6.75147	C14	
9.063	VV	1.85179	9.76185	7.23077	C15	
9.611	VB	2.34472	8.19963	7.69035	C16	
10.065	VV	2.53243	8.48848	8.59859	C17	
10.123	VV	2.21563	8.38821	7.43406		Pristane
10.501	VV	5.23318	4.19572	8.78279		C18
10.530	VV	2.90404	7.26713	8.44160		Phytane
11.176	VV	3.78808	5.91992	8.97005		C19
11.643	VV	11.68911	2.20219	10.29666		C20
12.091	VV	30.02251	1.08328	13.00912		C21
12.568	VV	22.73989	1.35323	12.30892		C22
12.923	VV	28.37482	1.17836	13.37426		C23
13.335	VV	84.84893	6.50954e-1	22.09311		C24
13.730	VV	54.43475	8.12941e-1	17.70089		C25
14.101	VV	12.80995	2.28478	11.70718		C26
14.469	VV	21.36765	1.53267	13.09982		C27
14.825	VV	23.64913	1.40686	13.30840		C28
15.150	VV	31.31776	1.16667	14.61496		C29
15.592	VV	20.82600	1.51252	12.59988		C30
15.982	VV	12.63616	2.07501	10.48808		C31
16.434	VV	5.73305	3.47180	7.96159		C32
17.009	VV	7.41993	2.55718	7.58963		C33
17.529	VV	2.56380	4.42037	4.53318		C34
18.316	BV	6.02408e-1	47.98982	11.56378		C35
19.012		-	-	-		C36
19.932		-	-	-		C37
21.016		-	-	-		C38
22.443		-	-	-		C39
23.834		-	-	-		C40
Totals :				287.37013		

4. CONCLUSION

Although the controversy in the use of engine oil as the spiking fluid for bioremediation research intended for deployment in crude oil impacted soil treatment is justified on the basis that crude oil and engine oil are two different fluids. Notwithstanding the controversy, record has it that numerous published articles on

bioremediation made use of engine oil as the spiking fluid. This builds confidence that it can be used in determination of a remediation approach ability to remediate crude oil impacted soils. However, there is no record validating its use. This gap has been identified and also bridged by this study.

Does the readily unavailability of crude oil make the readily available engine oil a perfect substitute for determining a remediation approach ability to remediate crude oil impacted soils? This study has successfully addressed this question. There is presently no record where engine oil alongside crude oil has been deployed in a single research in a bid to validate the use of engine oil in place of crude oil; this gap was recognized in this study and a hypothesis was therefore developed to bridge the gap. Engine oil as well as crude oil were deployed in this study as spill simulation fluid and same remediation approach was applied on both. Even though analysis of soil samples spiked with engine oil and crude oil yielded different values of Total Petroleum Hydrocarbon (TPH) which was expected considering the difference in colouration observed in the TPH extract of the two samples during the TPH extraction process; both set ups experienced reduction in TPH levels following applied remediation approach. Thus, this research has shown or experimentally validated that in the absence of crude oil, engine oil can effectively be used for bioremediation research that can be replicated in the treatment of crude oil impacted soils. We can, therefore, conclude that in the absence of crude oil, engine oil can effectively be used for research of possible effective remediation approaches intended for deployment in remediation of crude oil impacted soils.

ACKNOWLEDGMENT

I am thankful to my supervisors (Associate Prof. S.E. Abrakasa, Prof. H.O. Nwankwoala and Prof. L.C. Osuji) who guided me in my PhD research with their helpful information, practical advice and ideas. I am also thankful to ACE-CEFOR

for organizing series of progress report presentation that kept me on track with insightful comments and wholesome criticism that helped me in successfully carrying out the research. I express my sincere gratitude to Shell Petroleum Development Company of Nigeria Limited (SPDC) for accepting me into her graduate internship program and to the management of the Ogoni Restoration Project (ORP) team of SPDC for retaining me after the internship program. Working with the ORP team gave me the practical experience I needed to successfully complete my research.

REFERENCES

- [1] Abioye, O.P., Agamuthu, P., and Abdul Aziz, A.R., (2012). Biodegradation of Used Motor Oil in Soil Using Organic Waste Amendments. *Biotechnology Research International*. 2012: Article ID 587041, 8 pages.
- [2] Adenipekun, C.O., and Isikhuemhen, O.S., (2008). Bioremediation of Engine Oil Polluted Soil by the Tropical White Rot Fungus, *Lentinus Squarrosulus* Mont. *Pakistan Journal of Biological Sciences*. 12(12): 1634-1637.
- [3] Agarry, S.E., and Ogunleye, O.O., (2012). Box-Behnken Design Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Spent Engine Oil Using Biostimulation Strategy. *International Journal of Energy and Environmental Engineering*. 3(31): 1-14.
- [4] Mohammad, R.G., Mohammad, J.N., Muhammad, I.L., and Hamzat, A., (2022). Stimulated Bioremediation of Soil Contaminated with Spent Engine Oil Using Organic Wastes. *Science World Journal* 17: 308-314.
- [5] Ogbeh, G.O., Tsokar, T.O., and Salifu, E., (2019). Optimization of Nutrients Requirements for Bioremediation of Spent-Engine Oil Contaminated Soils. *Environ. Eng. Res.* 24(3): 484-494.