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OPTIMIZATION OF BIOSTIMULANTS REQUIREMENTS FOR ENHANCED BIOREMEDIATION OF CRUDE OIL SLUDGE POLLUTED SOILS

Chika Onu, C.I.O. Kamalu, J.C. Obijaku, M.S. Nwakaudu
Department of Chemical Engineering
Federal University of Technology, Owerri

E.E. Anyanwu
Department of Mechanical Engineering
Federal University of Technology, Owerri

Abstract: **The study was aimed at investigating the optimal requirement of biostimulants in the bioremediation of crude oil sludge polluted soils. The contaminated environment was simulatedwith 1.0kg crude oil sludge on 2.0kg soil and uniformly homogenized. The polluted soils were then simulated with NPK, Cowdung and Moringaleaf powder based on total biostimulant to soil ratio of 0:1 to 0.75:1. These biostimulants were applied individually and in varying combinations to the polluted soils in three distinct segments (1, 2, 3) corresponding to the total biostimulant to soil ratios 0.25:1, 0.50:1 and 0.75:1 respectively while the control experiment which proceeded on natural attenuation was 0:1. Results of the experiments which lasted for 92 days showed marked reduction in Total Petroleum Hydrocarbon (TPH) content in the biostimulated experiments, E1 to E30. The control experiment, E31, which was non-biostimulated showed minimal reduction in TPH throughout the experiment due to the absence of added nutrients. In the single biostimulant application, moringa leaf powder (MLP) was found to give the best degradative performance at an optimal biostimulant to soil ratio of 2.04:1corresponding to 99.9957% bioremediation of the polluted soil being investigated. This result justifies the introduction of MLP as a suitable biostimulant for bioremediation studies and a possible substitute for NPK which is very expensive and not readily available for use. In the double biostimulant combination category, NPK + mlp mixing resulted in the best degradation efficiency. The optimum extent of degradation was 100% at optimum biostimulant to soil ratio of 2.04:1. The optimum extent of remediation for combination of all three biostimulants (NPK + CD + MLP) were found to be 99.15% at optimal biostimulant to soil ratio of 2.04:1.**

I. INTRODUCTION

Hydrocarbon exploration activities orchestrated by rapid industrialization of crude oil producing countries like Nigeria have resulted in the grandiose pollution of the environment with diverse consequences on human health and the ecosystem. Pollution by crude oil and its components have become a problem of enormous magnitude worldwide (Liu et al., 2010).

Over 450 thousand tons of hazardous toxic wastes are discharged into environment on a global scale (Tiwari and Singh, 2014) which negatively affects the soil nutrients, microbial population of the soil and human health in general. It affects the ecosystems and biological activities by changing the parameters such as pH, moisture content, and aeration level of soil with introduction of toxicity (Bepkoskiet al., 2011, and Megharajet al., 2011).

Enormous quantities of crude oil sludge are being generated during crude oil production from oil wells, cleaning of crude oil storage tanks, processes of crude oil refining and separation processes at crude oil ocean terminals before export (Liu et al., 2010; Ayotamunoet al, 2011;Nkenget al., 2012 and Moraiset al., 2014). Global production of crude oil sludge has been reported to be about 60 million tons a year (Al-Shiaaniet al., 2021). A petroleum refinery with a production capacity of 105,000 drums per day generates approximately 50 tons of oily sludge per year (Olufemi and Augustine, 2019).

Several components of the crude oil sludge are toxic, mutagenic and carcinogenic (Liu et al., 2010; Ayotamunoet al., 2011; Barnabas et al., 2013 and Singh and Chandra, 2014).Crude oil sludge is mainly generated during the production, refining, storage and transportation of petroleum and includes mud from the drilling process, waste oil in the well, emulsified solids created during the crude oil refinery process and sediment in the storage tank (Deng et al., 2015; Vivanaet al., 2015; Wang et al., 2017 and Kunlong and Juan 2020).

Crude oil sludge is an emulsion of water/oil solid particles that contains 30% - 80% oil, 30% - 50% water and 10% - 20% solid particles (Jasmine and Mukherji, 2015). The Petroleum Hydrocarbons content of crude oil sludge are in the range of 40% - 52% alkanes, 28% - 31% aromatics, 8-10% asphaltenes and 7-22.4% resins by mass (Bezza et al., 2015 and Al-Shiaaniet al., 2021).

Crude oil sludge has been classified as a dangerous environmental pollutant by the United States Environmental Protection Agency. In Nigeria, crude oil sludge has been designated as hazardous pollutant by the Department of Petroleum Recourses (DPR) in Nigeria (DPR, 2002). When crude oil sludge eventually finds its way into the environment by improper disposal, the environment is therefore contaminated. Most of the crude oil sludge generated by the multinational oil companies, refineries, and petrochemicals in the Niger Delta region of Nigeria, are deliberately disposed into the environment without any form of treatment and hence results to the contamination of the environment. Petroleum hydrocarbons in the crude oil sludge over a passage of time migrate downwards from the topsoil layer through the subsoil thereby contaminating both the subsoil and underground water bodies. At the same time, by the environmental action of heat and wind, the lighter hydrocarbon components of the sludge get vaporized and blown into the atmosphere thereby polluting surrounding air.

Crude oil sludge therefore poses a big threat to the environment as well as to mankind (Rudyk, 2018). Crude oil sludge when inadequately disposed to the environment, contaminates the soil, causing loss of the soil fertility nutrients which leads to stunted growth of plants and over a passage of time pollutes the underlying groundwater environment (Usehet al., 2019). The environmental impact of crude oil sludge is a major problem facing the people of the Niger Delta region who are predominantly farmers and fishers (Inamet al., 2016).

It is therefore absolutely imperative that crude oil sludge be treated to avert its environmental hazards. Several physical and chemical mitigation methods have been documented for the cleanup of crude oil sludge contaminated soils; such as incineration, solvent extraction, chemical oxidation, soil washing, scooping and thermal desorption(Das and Chandran, 2010; Udeet al., 2013 and Ukpaka and Amadi, 2016). It is important to note that these physicochemical methods of crude oil sludge impacted soil mitigation are very expensive and not environmentally friendly, due to hazards associated with chemical additives and they do not completely remove the pollutants (Ukpaka and Amadi, 2016; and Okoro and Adoki, 2014). It is therefore against this backdrop that the need for a cheap, economic, and environmentally friendly mitigation approach to decontaminate crude oil sludge polluted soil has become absolutely inevitable. Environmentally friendly mitigation approach based on biological treatment of petroleum hydrocarbon wastes which gives a reliable, simple and cheap technologies is preferred over the conventional physicochemical methods (Ramirez et al., 2015 and Usehet al., 2019).

Bioremediation is a prominent method of cleaning hydrocarbon impacted soils by employing the degradative capabilities of microorganisms which accelerates the rate of the substrate degradation by subduing those factors that retard hydrocarbon degradation activities (Bijayet al; 2012). Heterotrophic bacteria and fungi have demonstrated high degradative capabilities to metabolize hydrocarbon pollutants in soil (Unimke et al., 2018). It has equally been affirmed that the metabolic capability of microorganisms, when properly utilized, has the possibility to mineralize hydrocarbon pollutants in soil (Hou and Al-Tabbaa 2014).

In Nigeria, it has been reported by Ayotamunoet al (2011) that a huge amount of crude oil sludge is generated during the cleanup of crude oil storage tanks and pre-export processing activities of crude oil at the ocean terminals. These values show the magnitude and the need for applying effective remediation technologies. Although, awareness of prevention and sustainable development practices continues to grow, industrialization of developing countries as well as improper disposal of petroleum products, ensures that contaminated sites remain a continual environmental problem.Accordingly, there is a critical and urgent need to develop and implement effective bioremediation of contaminated soils to reduce the threats caused by such contaminants.

II. MATERIALS AND METHODS

The major materials are soil, crude oil sludge, NPK fertilizer, Cow Dung (CD) and Moringa Leaf Powder (MLP). The soil was collected from a hydrocarbon unimpacted area of Bodo Community in Gokana Local Government Area of Rivers State, Nigeria. The soil was collected at a depth of 20cm at different areas of the sampling site, as the highest concentration of organic matter and microorganisms are found in this layer (Mann, 2008). The collected soil sample in a 50kg raffia grade bag was then transported to the experimental set-up site. Crude oil sludge sample was collected from the Nigerian Agip oil company flow station at Oshie town, Ahoada West Local Government Area of Rivers State, Nigeria. The sludge was gotten from the process of crude oil storage tank periodic cleaning. The collected crude oil sludge sample in 3 twenty litres containers was then transported to the experimental set-up site. NPK (20:10:10) fertilizer sample, sealed in a 50kg raffia bag was purchased from the Agricultural Development Program (ADP) office in Rumuodamaya, Obio/Akpor Local Government Area of Rivers State, Nigeria. The NPK sample was then transported to the experimental set-up site. Cow Dung sample was collected from the former Port Harcourt Central Abattoir, Oginiba, Trans-Amadi industrial layer Port Harcourt, Rivers State, Nigeria. The collected Cow Dung sample in a 25kg raffia bag was transported to the experimental set-up site. Moringa leaf powder sample was purchased from the Rivers State University farm, Nkpolu-Oroworukwo, Port Harcourt. The Moringa Leaf Powder sample sealed in a 25kg biaxial oriented polypropylene (BOPP) bag was transported to the experimental set-up site. At the experimental site, 200g of the soil sample, 200ml of the crude oil sludge sample, 200g of Cow Dung sample and 200g of the Moringa Leaf Powder sample were collected in clean polyethylene terephthalate (PET) bottles and taken to AUSTINO Research and analysis Laboratory, 2 UPTH Road, off East West Road, Port Harcourt, for initial physicochemical parameters characterization before the experiment.

Experimental Design

The experiment was designed based on the total biostimulant to soil ratio for 31 experiments categorized in 3 distinct sections:

Section 1: Experiments E1, E2, E3, E4, E5, E6, E7, E8, E9, E10

Section 2: Experiments E11, E12, E13, E14, E15, E16, E17, E18, E19, E20.

Section 3: Experiments E21, E22, E23, E24, E25, E26, E27, E28, E29, E30.

Control Experiment: E31

The totalized weight of the biostimulants (NPK, CD, MLP) used in each experiment are 0.75kg, 1.5kg and 2.25kg respectively for sections 1, 2, and 3 experiments, as shown in Tables 1, 2 and 3.

III. EXPERIMENTAL PROCEDURE

Thirty-one buckets (7litres capacity each) were labelled E1 to E31 to which 2.0kg of soil was weighed and added to each of the 31 buckets.

1.0kg of crude oil sludge was weighed and added to each of the 31 soil samples. The contents of the buckets were properly homogenized and kept in a closed room.

Five (5) hours after the pollution of the soil samples, 50g of each set of the polluted soil samples were collected in clean polyethylene terephthalate (PET) bottles and taken for initial analysis of TPH and TBC.Thereafter the polluted soils were biostimulated with the biostimulants in diverse variations in accordance with the experiment design as shown in Table 4.

Table 1: Experimental Design based on coded Ratios for Section 1

Experimental Run	Total Biostimulant Used	Total Biostimulant to Soil Ratio	Polluted Soil (PS) 2kg Soil + 1.0kg COS	Fraction o Biostimulants Used		
				NPK	CD	MLP
E1	0.75	0.25	PS1	1/3	1/3	1/3
E2	0.75	0.25	PS2	2/3	1/3	0/3
E3	0.75	0.25	PS3	2/3	0/3	1/3
E4	0.75	0.25	PS4	3/3	0/3	0/3
E5	0.75	0.25	PS5	1/3	2/3	0/3
E6	0.75	0.25	PS6	0/3	2/3	1/3
E7	0.75	0.25	PS7	0/3	3/3	0/3
E8	0.75	0.25	PS8	1/3	0/3	2/3
E9	0.75	0.25	PS9	0/3	1/3	2/3
E10	0.75	0.25	PS10	0/3	0/3	3/3

Table 2: Experimental Design based on coded Ratios for Section 2

Experimental Run	Total Biostimulant Used	Total Biostimulant to Soil Ratio	Polluted Soil (PS) 2kg Soil + 1.0kg COS	Fraction of Biostimulants Used		
				NPK	CD	MLP
E_{11}	1.50	0.50	PS_{11}	$^{1}/_{3}$	$^{1}/_{3}$	$^{1}/_{3}$
E_{12}	1.50	0.50	PS_{12}	$^{2}/_{3}$	$^{1}/_{3}$	$^{0}/_{3}$
E_{13}	1.50	0.50	PS_{13}	$^{2}/_{3}$	$^{0}/_{3}$	$^{1}/_{3}$
E_{14}	1.50	0.50	PS_{14}	$^{3}/_{3}$	$^{0}/_{3}$	$^{0}/_{3}$
E_{15}	1.50	0.50	PS_{15}	$^{1}/_{3}$	$^{2}/_{3}$	$^{0}/_{3}$
E_{16}	1.50	0.50	PS_{16}	$^{0}/_{3}$	$^{2}/_{3}$	$^{1}/_{3}$
E_{17}	1.50	0.50	PS_{17}	$^{0}/_{3}$	$^{3}/_{3}$	$^{0}/_{3}$
E_{18}	1.50	0.50	PS_{18}	$^{1}/_{3}$	$^{0}/_{3}$	$^{2}/_{3}$
E_{19}	1.50	0.50	PS_{19}	$^{0}/_{3}$	$^{1}/_{3}$	$^{2}/_{3}$
E_{20}	1.50	0.50	PS_{20}	$^{0}/_{3}$	$^{0}/_{3}$	$^{3}/_{3}$

Table 3: Experimental Design based on Coded Ratios for Section 3

Experimental Run	Total Biostimulant Used	Total Biostimulant to Soil Ratio	Polluted Soil (PS) 2kg Soil + 1.0kg COS	Fraction of Biostimulants Used		
				NPK	CD	MLP
E_{21}	2.25	0.75	PS_{21}	$^{1}/_{3}$	$^{1}/_{3}$	$^{1}/_{3}$
E_{22}	2.25	0.75	PS_{22}	$^{2}/_{3}$	$^{1}/_{3}$	$^{0}/_{3}$
E_{23}	2.25	0.75	PS_{23}	$^{2}/_{3}$	$^{0}/_{3}$	$^{1}/_{3}$
E_{24}	2.25	0.75	PS_{24}	$^{3}/_{3}$	$^{0}/_{3}$	$^{0}/_{3}$
E_{25}	2.25	0.75	PS_{25}	$^{1}/_{3}$	$^{2}/_{3}$	$^{0}/_{3}$
E_{26}	2.25	0.75	PS_{26}	$^{0}/_{3}$	$^{2}/_{3}$	$^{1}/_{3}$
E_{27}	2.25	0.75	PS_{27}	$^{0}/_{3}$	$^{3}/_{3}$	$^{0}/_{3}$
E_{28}	2.25	0.75	PS_{28}	$^{1}/_{3}$	$^{0}/_{3}$	$^{2}/_{3}$
E_{29}	2.25	0.75	PS_{29}	$^{0}/_{3}$	$^{1}/_{3}$	$^{2}/_{3}$
E_{30}	2.25	0.75	PS_{30}	$^{0}/_{3}$	$^{0}/_{3}$	$^{3}/_{3}$

After pollution of the soil and biostimulation, each set of the polluted soil samples were collected periodically on: Days 4, 8,22, 36, 50, 64,78,92 and tested in the laboratory for Total petroleum hydrocarbon (TPH) and Total bacteria count (TBC).

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Table 4: Experimental Design Based on Actual Values

Experimental Run	Polluted Soil	Fraction of Biostimulants and Used (kg)		
		NPK	CD	MLP
E_1	2kg Soil + 1.0kg COS	0.25	0.25	0.25
E_2	2kg Soil + 1.0kg COS	0.50	0.25	0
E_3	2kg Soil + 1.0kg COS	0.50	0	0.25
E_4	2kg Soil + 1.0kg COS	0.75	0	0
E_5	2kg Soil + 1.0kg COS	0.25	0.50	0
E_6	2kg Soil + 1.0kg COS	0	0.50	0.25
E_7	2kg Soil + 1.0kg COS	0	0.75	0
E_8	2kg Soil + 1.0kg COS	0.25	0	0.50
E_9	2kg Soil + 1.0kg COS	0	0.25	0.50
E_{10}	2kg Soil + 1.0kg COS	0	0	0.75
E_{11}	2kg Soil + 1.0kg COS	0.50	0.50	0.50
E_{12}	2kg Soil + 1.0kg COS	1.0	0.50	0
E_{13}	2kg Soil + 1.0kg COS	1.0	0	0.50
E_{14}	2kg Soil + 1.0kg COS	1.50	0	0
E_{15}	2kg Soil + 1.0kg COS	0.50	1.0	0
E_{16}	2kg Soil + 1.0kg COS	0	1.0	0.50
E_{17}	2kg Soil + 1.0kg COS	0	1.50	0
E_{18}	2kg Soil + 1.0kg COS	0.50	0	1.0
E_{19}	2kg Soil + 1.0kg COS	0	0.50	1.0
E_{20}	2kg Soil + 1.0kg COS	0	0	1.50
E_{21}	2kg Soil + 1.0kg COS	0.75	0.75	0.75
E_{22}	2kg Soil + 1.0kg COS	1.50	0.75	0
E_{23}	2kg Soil + 1.0kg COS	1.50	0	0.75
E_{24}	2kg Soil + 1.0kg COS	2.25	0	0
E_{25}	2kg Soil + 1.0kg COS	0.75	1.50	0
E_{26}	2kg Soil + 1.0kg COS	0	1.50	0.75
E_{27}	2kg Soil + 1.0kg COS	0	2.25	0
E_{28}	2kg Soil + 1.0kg COS	0.75	0	1.50
E_{29}	2kg Soil + 1.0kg COS	0	0.75	1.50
E_{30}	2kg Soil + 1.0kg COS	0	0	2.25

3.1 Determination of Total Petroleum Hydrocarbon (TPH)

20g of the air-dried sample was measured and placed in an extracting timble and extracted in a soxhlet extractor using n-hexane as the extraction solvent. The oil extract in n-Hexane was thereafter distilled to recover the solvent from the oil to obtain the crude extract. A mixture of 20ml of n-hexane and 0.5g of the crude extract was then poured into a glass column of silica gel bed, for the cleaning of the crude extract. The crude extract which was dissolved in n-hexane eluted through the silica gel bed and collected in a beaker and allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place.10µL of the concentrated sample that eluted the glass column was then injected into GC-FID column for compound separation detection and summation of Total petroleum hydrocarbons.

IV. RESULTS AND DISCUSSION

The Results of Bioremediation Laboratory Experiments are presented in Tables, 5, 6 and 7

Table 5: Total Petroleum Hydrocarbon (TPH) Results for Section 1 Experiment (E_1 - E_{10})

Days	TPH E_1 (mg/kg)	TPH E_2 (mg/kg)	TPH E_3 (mg/kg)	TPH E_4 (mg/kg)	TPH E_5 (mg/kg)	TPH E_6 (mg/kg)	TPH E_7 (mg/kg)	TPH E_8 (mg/kg)	TPH E_9 (mg/kg)	TPH E_{10} (mg/kg)
0	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692
4	35887.846	35911.441	35799.995	36001.773	35876.056	36011.091	36380.123	35889.911	36001.665	35500.445
8	32533.713	32317.956	33340.668	34110.256	33751.133	33500.129	35005.781	337571.865	33150.889	33501.997
22	25398.786	21.533.462	22732.904	26,270.557	25000.094	25145.949	28004.991	25010.099	27009.989	28011.985
36	18115.392	19033.641	19532.925	23001.123	21106.382	21077.906	24011.170	21288.631	23514.949	25110.192
50	15415.984	16333.476	16670.655	20503.345	18406.256	19009.775	21504.478	18588.304	20814.249	23250.078
64	13613.659	14531.327	15000.134	18657.751	16604.136	17345.322	20004.560	16786.941	19012.139	20800.766
78	12039,283	12957.463	14156.206	17694.521	15030.754	16569.150	19150.883	15212.940	17438.187	19233.692
92	11666.781	11996.648	14064.045	17344.141	14033.086	16001.221	19001.709	15009.950	17050.268	19009.123

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Table 6: Total Petroleum Hydrocarbon (TPH) Results for Section 2 Experiment (E_{11} – E_{20})

Days	TPH E_{11} (mg/kg)	TPH E_{12} (mg/kg)	TPH E_{13} (mg/kg)	TPH E_{14} (mg/kg)	TPH E_{15} (mg/kg)	TPH E_{16} (mg/kg)	TPH E_{17} (mg/kg)	TPH E_{18} (mg/kg)	TPH E_{19} (mg/kg)	TPH E_{20} (mg/kg)
0	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692
4	35009.645	35022.989	35889.905	35987.674	35019.981	35591.773	35895.555	35020.419	35600.460	35555.345
8	26250.117	29375.751	32544.091	33751.595	31898.098	31308.915	32545.901	30999.916	32550.345	32566.437
22	15850.336	15015.591	15009.956	18750.012	17095.999	16252.659	18750.988	15833.345	18091.594	18761.435
36	10030.987	10988.236	10938.341	13771.180	12443.511	12599.907	15262.405	11690.595	13296.781	14894.128
50	6554.422	7505.668	7650.568	10098.175	10009.975	11052.376	12562.506	8990.192	10596.574	12194.311
64	4109.351	5410.778	6099.875	6607.798	6867.903	9250.147	10760.359	7685.195	9292.462	10392.756
78	3218.090	4550.667	4685.483	5067.670	6656.215	8225.755	9593.157	6111.465	7717.408	9360.257
92	3001.995	4061.347	4450.185	4737.790	5761.152	8099.518	9418.631	5995.193	7509.559	9019.709

Table 7: Total Petroleum Hydrocarbon (TPH) Results for Section 3 Experiment (E_{21} – E_{30}) and control, E31

Days	TPH E_{21}	TPH E_{22}	TPH E_{23}	TPH E_{24}	TPH E_{25}	TPH E_{26}	TPH E_{27}	TPH E_{28}	TPH E_{29}	TPH E_{30}	TPH E_{31} (mg

	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	/kg)
0	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692	36775.692
4	33765.125	35254.958	35011.843	31550.123	35009.815	33295.781	35011.870	30200.489	32590.691	32,509.875	35555.345
8	28903.111	30909.396	30295.664	27536.779	30869.709	27651.898	26259.980	25505.516	23750.622	25011.875	32566.437
22	15109.098	20122.946	15006.678	16433.539	15551.223	16250.775	16015.714	15156.786	12,509.228	14119.740	18761.435
36	8041.467	8104.216	8316.319	9501.986	9170.568	11250.356	11157.192	8376.544	7509.997	9083.605	14894.128
50	4552.937	5404.769	5616.391	7276.825	6370.906	6971.729	8457.139	5721.463	6478.238	6383.519	12194.311
64	3247.401	4099.814	4311.682	5971.511	4188.960	4996.351	7152.192	4290.910	5173.606	5081.267	10392.756
78	2412.932	3264.526	3476.474	5136.189	4119.376	4192.539	5578.163	3581.942	4299.917	4246.892	9360.257
92	1555.917	2806.385	2819.722	5089.907	3895.578	4117.531	5150.137	2724.516	4198.556	4038.9218	9019.709

4.1 Bioremediation Laboratory Results

The results of the 92 days bioremediation experiment are presented in Tables5-7. The results showed marked reduction in concentration of Total Petroleum Hydrocarbon (TPH) in the biostimulated experiments, E1 to E30, including the non-biostimulated experiment, E31 (control), which proceeded on natural attenuation. It was observed that the rate of substrate reduction increased with increase in the quantity of biostimulants added. The first week of the bioremediation experiment recorded low reduction in TPH in all experimental samples, due to microbial lag phase effect. By the 64th day of the experiment which is observed to be the climax of the microbial exponential phase, over 47.5%, 70% and 80% reduction have occurred in sections 1, 2 and 3 experiments respectively. This observation was as a result of the increase in the population of microbes responsible for crude oil sludge degradation.

4.2 Single Biostimulant Combination Efficiencies

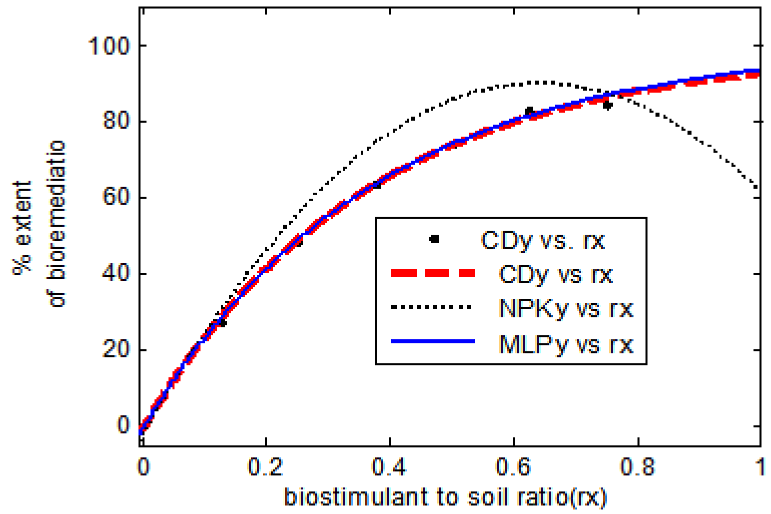

Figure 1A: Individual biostimulant extent of bioremediation versus biostimulant to soil ratio

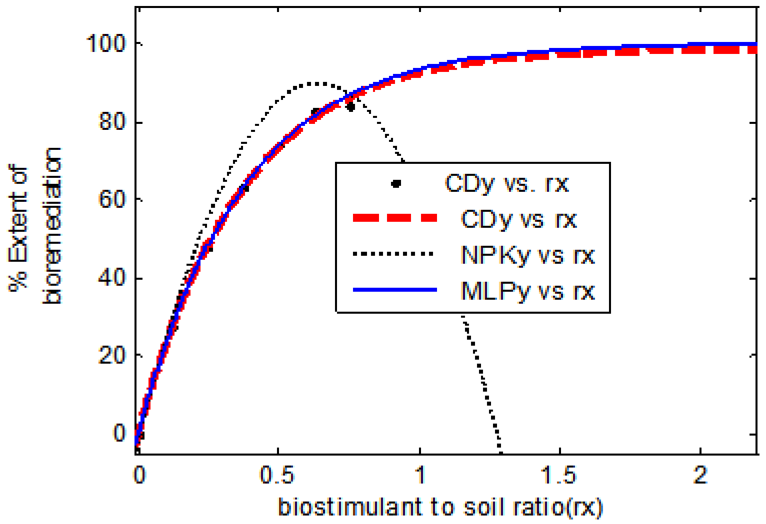

Figure 1B: Predictive individual biostimulant extent of bioremediation versus biostimulant to soil ratio

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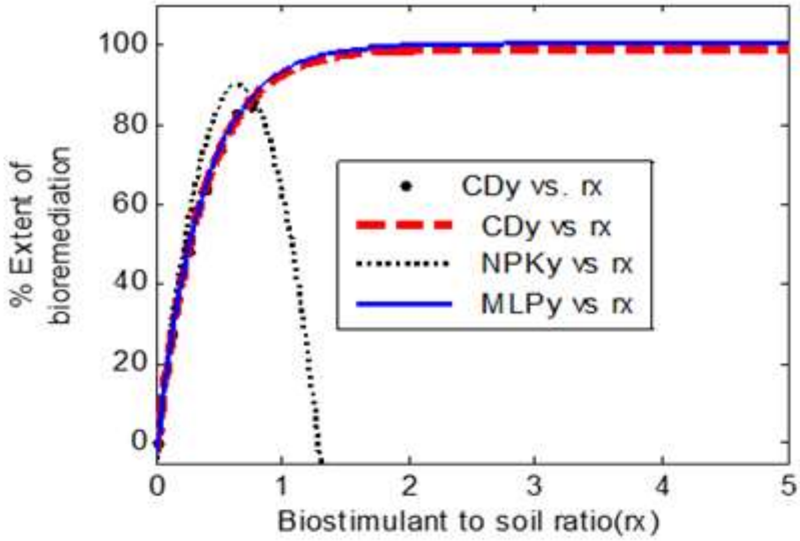

Figure 1C: Predictive individual biostimulantextent of bioremediation versus biostimulant to soil ratio

The percentage extent of bioremediation of the single biostimulant combinations (experiments: E4; E7; E10; E14; E17; E20; E24; E27; E30) are presented in Table 8 with their profile variations as shown in Figures 1A, 1B, 1C.

Table 8: Percentage Extent of Bioremediation for Single Biostimulant

Ratio, rx	0	0.125	0.25	0.375	0.50	0.625	0.75
NPK (%)	0	33	53	76	85	86.7	87.12
CD (%)	0	31	48.33	65	74.5	81	85.99
MLP (%)	0	32	48.31	66	76	82.50	89.02

Figure 1A shows the comparative optimum profiles for the individual biostimulants, NPK; CD and MLP on a biostimulant to soil ratio of 0:1 to 0.75:1. For the NPK biostimulant, it was found that 0.642:1 is the optimum biostimulant to soil ratio. At this optimum ratio, the optimum extent of degradation was deduced to be 90.138%. Beyond the ratio of 0.642:1, the NPK biostimu lantefficiency was found to be retrogressive, while the CD and MLP continues effectively in their degradative capabilities. Hence, the profiles were projected beyond the experimental values to predict the optimum values for CD and MLP. At biostimulant to soil ratio of 1:1, the extent of remediations for MLP and CD were respectively 93.4643% and 92.6244%. When the profiles were further, projected outside the experimental values, the optimum ratio of the MLP and CD biostimulants were found to be 2.04:1 as shown in figure 1B which corresponds to optimum extent of degradation 99.9957% for MLP and 98.7682% for CD. In figure 1C, the profiles were further extended to ratio 5:1, but the extent of degradations for MLP and CD remained the same: F(2.04) = F(5.0) = 99.9957% and F(2.04) = F(5.0) = 98.7682% respectively. Hence, the best choice of single biostimulant is MLP (99.9957%) on biostimulant to soil ratio of 2.04:1 followed by CD (98.7682%) on biostimulant to soil ratio of 2.04:1 and NPK (90.138%) on biostimulant to soil ratio of 0.642:1. This result has revealed within its context, the suitability of moringa leaf powder (MLP) as a biostimulant, hence enriching the data base of possible biostimulants for bioremediation studies of hydrocarbon polluted soils.

4.3 NPK and CD Combinations

The extent of remediation for NPK and CD combinations (experiments: E2, E5, E12, E15 and E22; E25) are presented in Table 9 and Figure 2A and 2B.

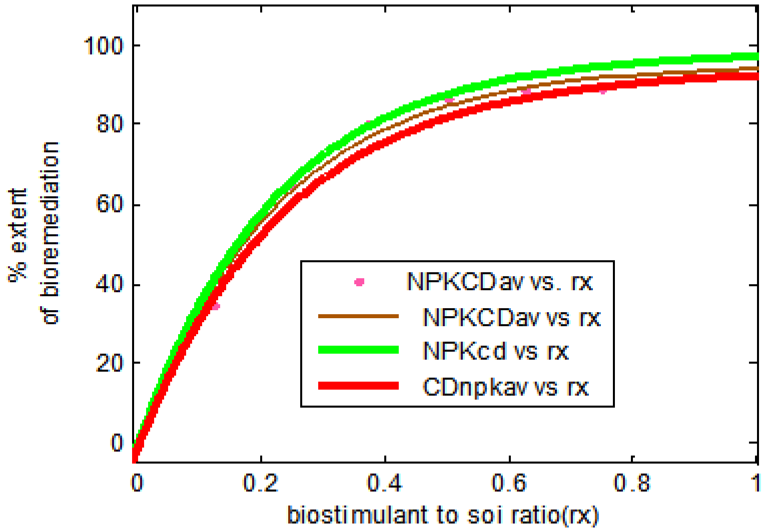

Figure 2A: Extent of bioremediation versus biostimulant to soil ratio for NPK and CD combinations

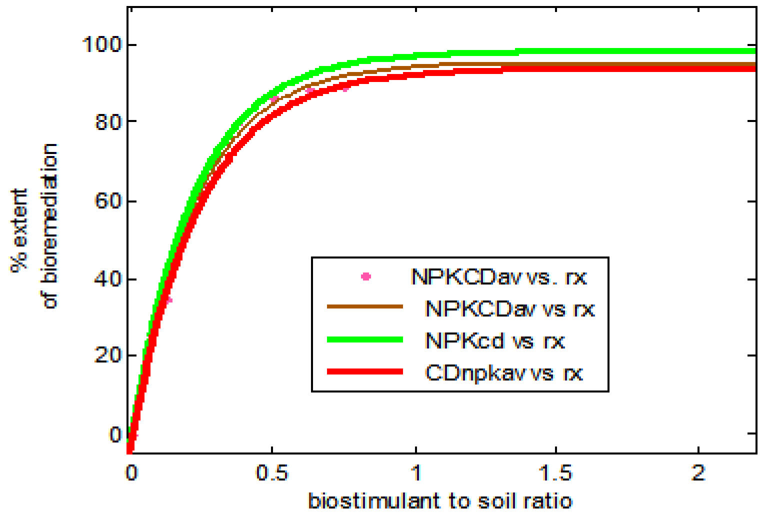

Figure 2B: Predictive extent of bioremediation versus biostimulant to soil ratio for NPK and CD combinations

Table 9: Extent of Remediation for NPK and CD Combinations

Ratio, rx	0	0.125	0.25	0.375	0.5	0.625	0.75
NPK + cd (%)	0	37	67.38	82	88.96	92	92.57
CD + npk (%)	0	30	61.84	77.5	84.33	86	85.99
NPK.CD average (%)	0	34.5	64.61	80	86.65	88.5	89.18

Figure 2A is a variation profile showing the various combinations of NPK and CD biostimulants at varying biostimulant to contaminated soil ratio in the experimental range of 0:1 to 0.75:1. Figure 2B is a predictive profile of the NPK and CD biostimulants beyond the experimental design of 0:1 to 0.75:1. From Figure 2A, the various combinations: NPK + cd and CD + npk showed potential degradative capabilities beyond the experimental ratio limit of 0.75:1. Hence, both profiles were taken beyond 0.75:1 to a biostimulant to soil ratio of 2.04:1 where the optimum for the various biostimulant combinations occurred. The optimum extent of bioremediation for NPK + cd and CD + npk were respectively found to be:

NPK + cd : F(2.04) = 98.1892%
CD + npk : F(2.04) = 93.6437%

The average of both combination gave an optimum value of F(2.04) = 95.11%. Hence, for effective bioremediation of crude oil sludge polluted soils, NPK and CD should be combined in the biostimulant to soil ratio of 2.04:1 (4.08kg NPK + 2.04 CD) to give 98.1892% remediation of the crude oil sludge polluted soils.

4.4 NPK and MLP Combinations

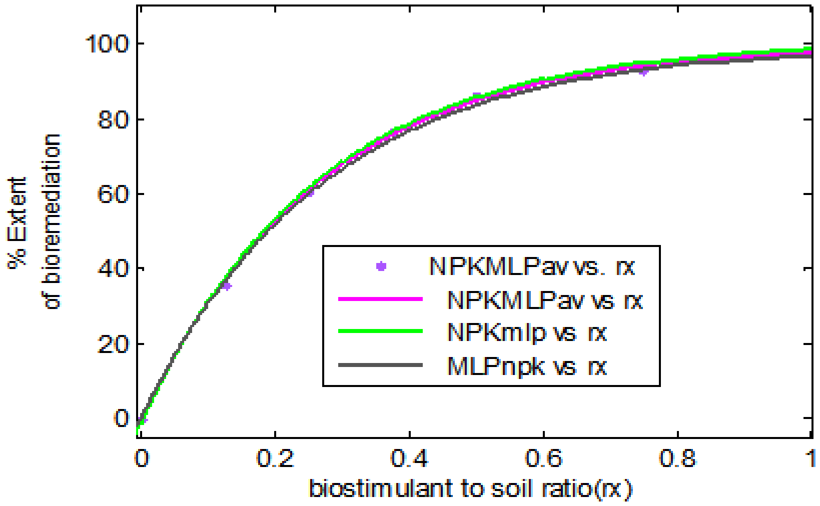

Figure 3A: Extent of bioremediation versus biostimulant to soil ratio for NPK and MLP combinations

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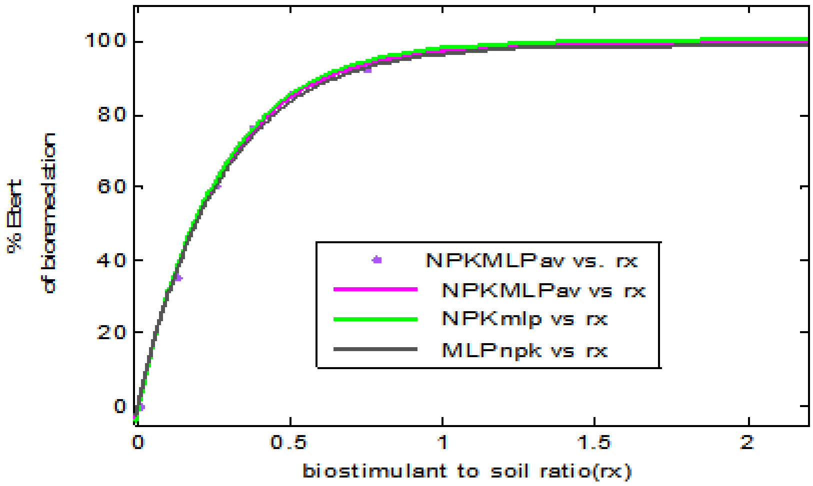

Figure 3B: Predictive extent of bioremediation versus biostimulant to soil ratio for NPK and MLP combinations

The results of the extent of remediation of the experiments (E3, E8, E13, E18 and E23; E28), for NPK and MLP combinations are shown in Table 10 and Figures 3A and 3B.

Table 10: Extent of Bioremediation for NPK and MLP Combinations

Ratio, rx	0	0.125	0.25	0.375	0.50	0.625	0.75
NPK + mlp	0	34	61.76	78	87.90	91	92.33
MLP + npk	0	37	59.19	74	83.69	89.5	92.59
NPK.CD average	0	35.5	60.48	76.50	85.80	90.5	92.46

Figure 3A depicts the trend and pattern for NPK and MLP combinations with respect to the biostimulant to contaminated soil ratio range of 0:1 to 0.75:1. While Figure 3B shows same combination trends in predicting the extent of bioremediation beyond the experimental biostimulant to soil ratio values. The various combination modes for NPK and MLP showed increasing efficiency as the biostimulant to contaminated soil ratio increases, as shown in Table 10.Atbiostimulant to soil ratio of 0.75:1, NPK +mlp and MLP + npk remediated the polluted soil by 92.33% and 92.59% respectively. Both combinations showed potentials for further degradative capacities and hence were extended beyond the experimental values to predict the optimum extents of bioremediation as:

NPK + mlp: F(1.0) = 98.2208%; F(2.04) = 99.89%
MLP + npk: F(1.0) = 96.6586%; F(2.04) = 99.1176%

The average of both combinations gave an optimum of F(2.04) = 99.7876%. Hence, for effective bioremediation of crude oil sludge polluted soils, NPK and MLP should be combined in the biostimulant to soil ratio of 2.04:1 as 4.08kg NPK + 2.04kgMLP to give 99.89% bioremediation.

4.5 CD and MLP Combinations

The experimental results for the various combinations of CD and MLP (E6, E9; E16, E19 and E26, E29) are shown in Table 11 and Figures 4A and 4B.

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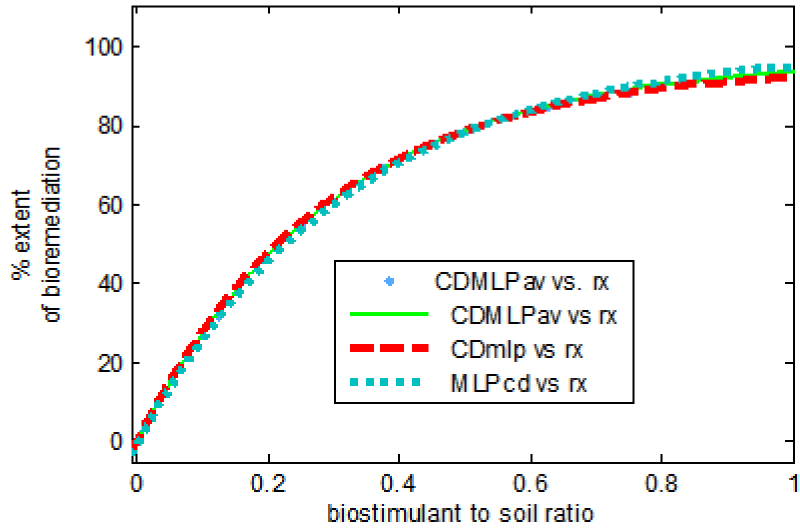

Figure 4A: Extent of bioremediation versus biostimulant to soil ratio for CD and MLP combinations

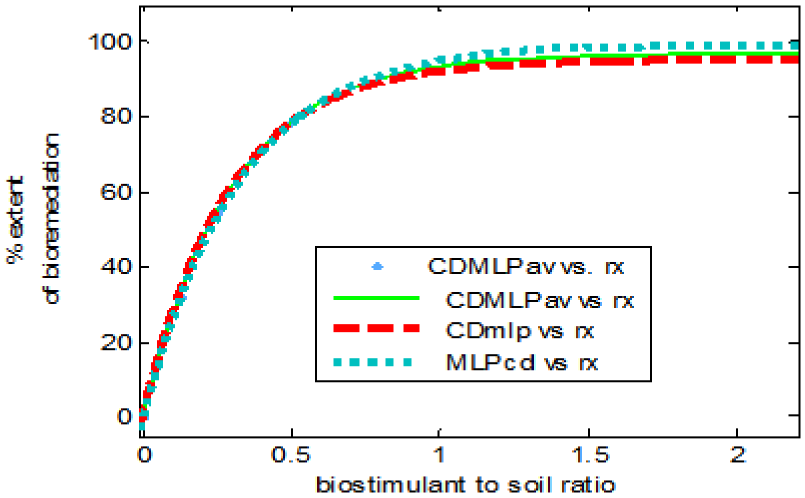

Figure 4B: Predictive profile for Extent of bioremediation versus biostimulant to soil ratio for CD and MLP combinations

Table 11: Extent of Bioremediation for CD and MLP Combinations

Ratio, rx	**0**	**0.125**	**0.25**	**0.375**	**0.50**	**0.625**	**0.75**
CD + mlp (%)	0	33	56.49	69	77.98	84	88.80
MLP + npk (%)	0	29	53.64	69	79.58	85	88.58

CD/MLP average (%)	0	32	55.065	69.5	78.78	85	88.69

Figure 4A is the CD and MLP combination profiles at varying biostimulant to soil ratio ranging from 0:1 to 0.75:1, while figure 4B is the prediction profile of the CD/MLP combination at biostimulant to soil ratios exceeding the experimental values. CD and MLP combinations were found to be effective in the bioremediation studies of crude oil sludge polluted soils as shown in Table 11 and Figure 4A. With over 88% remediation being achieved, and the profile pattern still indicates further degradation capabilities, hence the combinations were projected beyond the experimental values to predict the optimum extent of bioremediation. The optimum extent of remediation predicted were:

CD + mlp : F(1) = 92.2623%; F(2.04) = 95.11%
MLP + cd : F(1) = 95.051%; F(2.04) = 99.4%

The average of both combinations gave a predicted optimum value of F(2.04) = 96.92%.

Hence for effective remediation of crude oil sludge polluted soils, CD and MLP should be combined in the biostimulant to soil ratio of 2.04:1 (4.08kg MLP + 2.04kg CD) to obtain 99.4% extent of remediation.

4.6 All Biostimulants (NPK + CD + MLP) Combinations

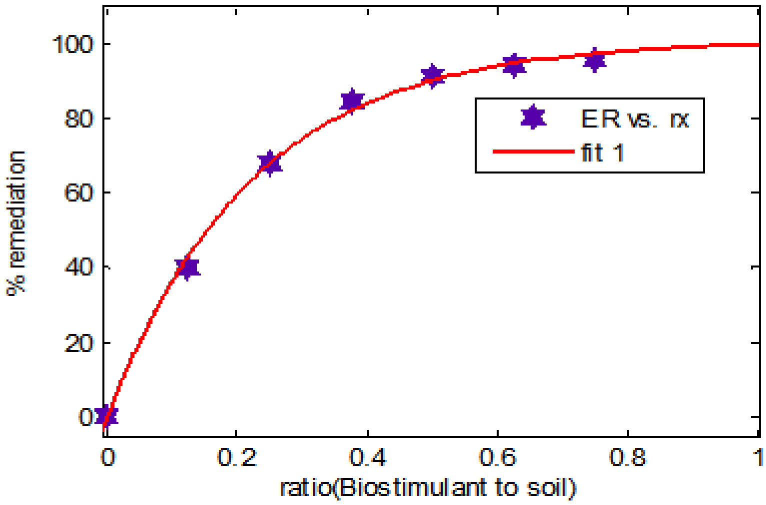

Figure 5: Extent of bioremediation versus biostimulant to soil ratio for all biostimulants (NPK + CD + MLP) combinations.

The results of the various experiments (E1; E11 and E21) where all the component biostimulants were applied in accordance with the biostimulant to soil ratio of 0:1 to 0.75:1 are presented in Table 12 with their degradative pattern profile as shown in Figures 5.

Table 12

Ratio, rx	**0**	**0.125**	**0.25**	**0.375**	**0.50**	**0.625**	**0.75**
Extent of NPK + CD + MLP	0	39.5	68.28	85.0	91.84	94.3	95.75

(%)							

V. CONCLUSION

The profile shows that the bioremediation of the crude oil sludge polluted soil increases as the biostimulant to soil ratio increases. At the ratio of 0.75:1, the extent of remediation was 95.75%, with the profile still showing capability of the biostimulants to further degrade the pollutants beyond the experimental values of 0:1 to 0.75:1. The profile was therefore extended outside the scope of the experiment to predict the optimum bioremediation values. The optimum values recorded was F(1.0) = 98.90%. Hence for effective bioremediation, all the biostimulants should be combined in a biostimulant to contaminated soil ratio of 1:1 (1.0kg NPK + 1.0kg CD + 1.0kg MLP) to obtain 98.90% remediation of the crude oil sludge polluted soils.

The biostimulants (NPK, CD, MLP) used in the remediation experiment were sufficient in nutrients such as Nitrogen, Phosphorus, Potassium required for basic microbial activities and as such are adequate for the stimulation of microbial activities for enhanced bioremediation studies. Higher rates of substrate degradation observed in the biostimulated samples were due to biostimulants enhancements that resulted in proliferation of microorganisms. As the total biostimulant to contaminated soil ratio increases, the rate of bioremediation of the sludge contaminated soils equally increases.

From the results of the experiment, the substrate reduction in the biostimulated samples were in the range of 48% to 95% compared to 6.31% in the control experiment and this observation establishes the fact that bioremediation of crude oil sludge polluted soils can proceed under natural attenuation but at much slower rates. Moringa leaf powder which was introduced by the present study as a biostimulant compared well with existing conventional biostimulants, hence could be a possible substitute for NPK (fertilizer) which is not in abundance, not readily available and very costly. For optimal usage of the biostimulants and efficient bioremediation process, the present study established that NPK and CD should be combined in the biostimulant to contaminated soil ratio of 2.04:1 (4.08kg NPK + 2.04kg CD) to give 98.1892% remediation of crude oil sludge polluted soils.

NPK and MLP should equally be combined in the ratio of 2.04:1 (4.08NPK + 2.04MLP) to give 100% remediation of the crude oil sludge polluted soils.CD and MLP should be combined in the ratio of 2.04:1 (4.08kg MLP + 2.04kg CD) to give 99.4% remediation of the crude oil sludge polluted soils. For single biostimulants application, the study revealed MLP as the best choice biostimulant on a biostimulant to soil ratio of 2.04:1 to give 99.9957% remediation; followed by CD to give 98.7682% remediation on biostimulant to contaminated soil ratio of 2.04:1 and NPK to give 90.138% remediation on a biostimulant to contaminated soil ratio of 0.642:1.

All biostimulants (NPK + CD + MLP) should be combined in the biostimulant to contaminated soil ratio of 2.04:1 (2.04kg NPK + 2.04kg CD + 2.04kg MLP) to give 99.9915% remediation of the crude oil sludge polluted soils. The study revealed an optimum biostimulant to contaminated soil ratio of 2.04:1 for efficient and effective bioremediation of sites heavily contaminated with crude oil sludge.

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