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Effect of Bacteria on Crude Oil Degradation in Loamy and Clay Soil for Water and Ethanol Biostimulant Extraction from *Bryophylum pinnatum* Leaf

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Abstract

The effect of bacteria on crude oil degradation in loamy and clay soil for water and ethanol biostimulant extraction from Bryophylum pinnatum leaf was investigated to ascertain the potential of the bacteria counts in the bioreactors sampled. At the progressive phase, the bacteria counts were more with bioreactors induced with ethanol solvent extract as the volume of the dosage increases compared to the bioreactors induced with the water extract. The research revealed that the bacteria growth was not induced by any factor at the progressive stage of the process, however a rapid increase in bacteria was experienced with increase in TPH degradation in each of the bioreactors. The biostimulant used in this investigation has revealed its potential in mitigating and restoration of a contaminated site by the application of solvent extraction techniques and approach of bioremediation concept. However, the bacteria counts were more on loamy soil with increase in ethanol application of solvent extraction compared to water application of solvent extracts, as well as in the case of clay soil, the same trend of characteristics was followed. But the bacteria count in clay soil was low compared to the loamy soil and this characteristic was attributed to the porosity of the soil type.

Keywords: Effect, bacteria, crude oil, degradation, loamy soil, water, ethanol, *Bryophylum pinnatum* leaf

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INTRODUCTION

Soil contaminated by petroleum hydrocarbons can affect the physical, chemical, and biological properties of soil [1–3]. The presence of hydrocarbons in soil affects microorganisms, reducing their number and activities [4, 5]. The bioavailability of hydrocarbon to degrading microbes is a critical factor in bioremediation, but strategies to enhance it are not fully optimized. No studies could be found in the open-access literature on the degradation of petroleum hydrocarbon of crude oil using *Bryophylum pinnatum* plants on clay and loamy soils [6].

This study investigated the degradation potential of crude oil contaminants using *Bryophylum pinnatum* plants for treatment. It focused on the effectiveness and reliability of *Bryophylum pinnatum* plant extracts for managing crude oil-contaminated soils. Understanding the nutrient

composition and microbial growth conditions by ensuring the practical feasibility of bioremediation were crucial steps toward sustainable and successful remediation efforts.

The bacterial growth and the inhibiting factors have been considered in various ways and concept for the purpose of drawing out conclusion for the best practice for effective bioremediation program [7–10]. Indeed, no conclusion has been made in this case, because of the complexity of the environmental conditions [11, 12]. In bioremediation, the bacterial growth was always monitored for the purpose of ensuring the progress of the remediation process, and in some cases where the program set-up has inhibited the tendency for remediation to occur may not be possible especially, when the active site of microbes is inhibited [13–15].

There is need to examine the trend of microbial characteristics during crude oil remediation as well as identify the constrain factors that induce the reaction mechanism [16]. The available nutrients obtained from the plant extract enhanced the performance of the microbes and its microbial growth kinetics [17–20]. The method of application of the plant extraction also may be attributing factors to the bacteria growth, because adding excess of the nutrients in most cases may result in causing menace in the microbial growth.

MATERIALS AND METHODS

Sample Collection

The clay soil was collected from a nearby river bank in Mgboushimini Community, Obio-Akpor local government area, Rivers State; and loamy soil was collected from Rivers State University. The loamy soil and *Bryophylum pinnatum* plants was collected from the Rivers State University demonstration farm and transported to Chemical Engineering laboratory, Rivers State University. Crude oil sample was collected from the defunct Department of Petroleum Resources (DPR), now known as Nigerian Upstream Petroleum Regulatory Commission (NUPRC) and transported to Chemical Engineering laboratory to be analyzed.

Bryophylum pinnatum Leaves Biostimulant Preparation

Freshly harvested *Bryophylum pinnatum* leaves were thoroughly washed with water, chopped into pieces and soaked in 5 l of water and alcohol for a fermentation period of 21 days. The leaves were filtered and disposed, the filtered water and ethanol were used as biostimulant for treatment of crude oil polluted soil. The characterization of the soil was done in the Department of Soil Science, Rivers State University, on the basis of particle size distribution of the soil.

Procedure for Total Bacterial Count (TBC) Analysis

Pour plating technique was used for the microbiological investigation and enumeration of heterotrophic bacteria and fungus. In order to do this, 0–1 ml of 10-fold diluted material was inoculated onto mineral salt agar (MSA) (hydrocarbon degraders), acidified streptomycin (1 mg/100 ml) (fungal), and nutrients agar (bacterial). The following components are present in grams per liter of distilled water in the mineral salt medium was described as well as modified as presented in in relationship to each compound and mass: NaCl 10 g, $MgSO_4.7H_2O$, 9.42 g, KCl 0.29 g, HPO_4 1.2 g, KH_2PO_4 0.83 g, $NaNO_2$ 0.42 g, Agar-Agar 16 g, pH 7.2 and 2 nill at gasoline/diesel. While the potato dextrose Agar plates were hatched at room temperature and tallied and described as colony firming units per gram (cfu/ml), the injected nutrient agar plates were incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

The outcome of results from the research is presented in Figures and Tables. Figures 1 and 2 show the microbial growth of the hydrocarbon utilizing bacterial counts for the various bioreactors with the addition of different dosages of water and ethanol from *Bryophylum pinnatum* leaves as biostimulants. The trend of hydrocarbon utilizing bacteria across treatment with different dosages during the lag phase with contact time of 0 day using 100, 200 and 300 ml water and ethanol biostimulant has been shown. At the progressive phase of 7 to 21 days of treatment, there was tremendous growth of bacteria counts

as shown in Tables 1 to 4. The treatment recorded an optimum increase of bacteria counts during the 28 and 35 days with different dosages of biostimulants which is interpreted as the stationary phase. The reduction of hydrocarbon in the media due to application of water and ethanol biostimulants resulted to high level of competitiveness among the microorganisms that makes them decline towards the 42 days of study (Figures 1 and 2). In other words, the decline of bacteria counts increases total organic carbon, phosphorus, and nitrogen cycling in low contaminated loamy soil.

Table 1. Bacteria count for water biostimulant on loamy soil.

	T3W (100 ml)	T4W (200 ml)	T5W (300 ml)	T1 Control
0	1036	1036	1036	1036
7	11480	20517	26193	2713
14	25116	27924	38011	4091
21	1634700	1953600	2076200	5122
28	20707000	2307000	26613000	6913
35	20707000	53176000	58416000	8016
42	100150	101060	147050	10712

Table 2. Bacteria count for ethanol biostimulant on loamy soil.

	T6E (100 ml)	T7E (200 ml)	T8E (300 ml)	T1 Control
0	1036	1036	1036	1036
7	15055	23807	29481	2713
14	28233	30517	4115	4091
21	17938	2150200	236140	5122
28	23141000	25396000	28119000	6913
35	23141000	25396000	28119000	8016
42	12537	20825	11803	1070

Table 3. Bacteria count for ethanol biostimulant on clay soil.

	T9E (100 ml)	T10E (200 ml)	T11E (300 ml)	T2 Control
0	1036	1036	1036	1036
7	8304	10360	11022	1527
14	11727	12416	13190	2196
21	1851200	273100	3854600	2813
28	21331000	35176000	58416000	3483
35	21331000	53176000	58416000	4107
42	12147	10817	10810	4616

Table 4. Bacteria Count for Water Biostimulant on Clay Soil.

	T12W (100 ml)	T13W (200 ml)	T14W (300 ml)	T2 Control
0	1036	1036	1036	1036
7	7092	8517	10015	1527
14	10137	10651	12035	2196
21	133500	157150	208470	2813
28	1934200	2095200	2462700	3483
35	1834200	2095200	2462700	4107
42	21730	15015	17006	4616

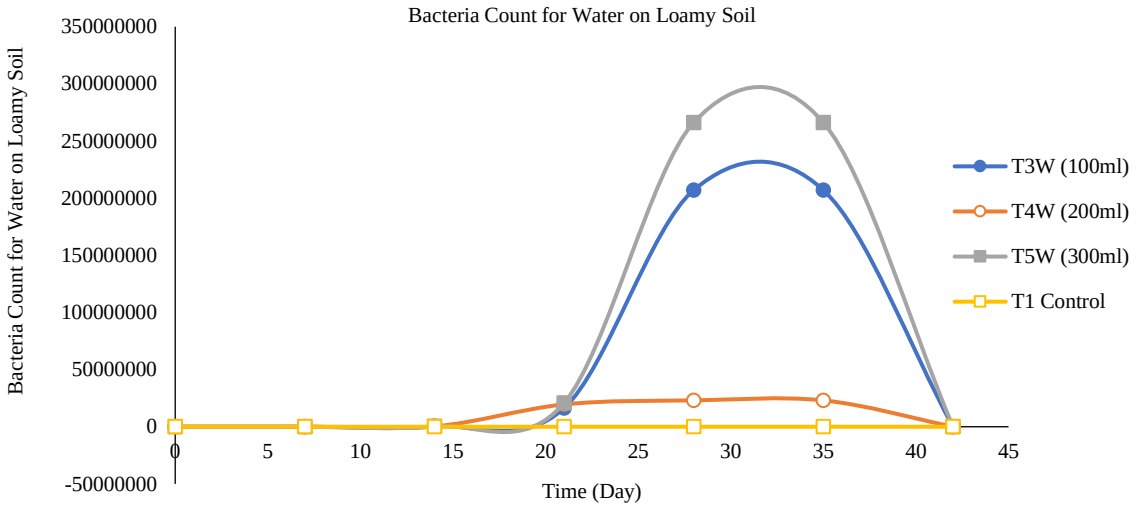

Figure 1. Effect of bacteria count in crude oil contaminated loamy soil treated with *Bryophylum pinnatum* Leaves from Water.

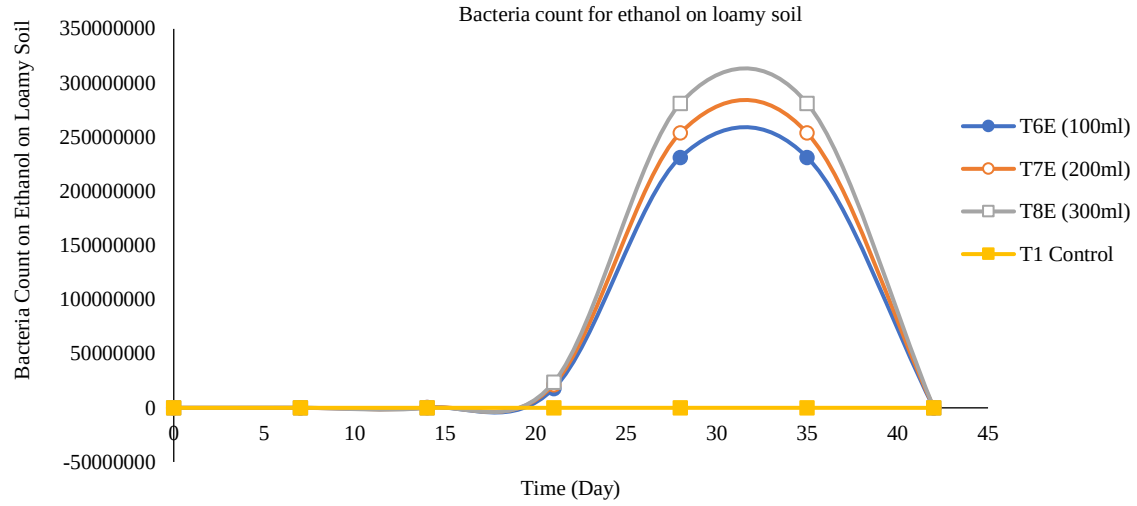

Figure 2. Effect of bacteria count in crude oil contaminated loamy soil treated with *Bryophylum pinnatum* leaves from Ethanol.

However, increase in time of remediation had multiplying effect on microbes with high reduction of hydrocarbon content in the sample as well as established on the process of microbial degradation of petroleum includes steps such as contact time with hydrocarbons that use them as source of energy. The biostimulant provided the needed nutrients for bacteria to thrive in hydrocarbon environment. Similar observation occurred in Figure 2 with different mix proportion of nutrients with peak of bacteria population at 35 days of treatment of 253.96×10^6 cfu/ml using 300 ml. The earlier stage of treatment with increase nutrients enhanced rapid bacteria growth that modulates the hydrocarbon content of crude oil in soil. Overall, it can be seen that ethanol biostimulant performed better on both clay and loamy soil.

Effect of Bacteria on Crude Oil Degradation in Clay Soil for Water and Ethanol Biostimulants

Figures 3 and 4 reveal the degradation rate of total petroleum hydrocarbon (TPH) using *Bryophylum pinnatum* leaves soaked in water and ethanol as biostimulants on clay soil. Bacteria growth rate before application of water and ethanol biostimulant was 10.36×10^2 cfu/ml. With 100 ml of ethanol biostimulant showed increase in bacteria growth 213.31×10^2 cfu/ml and the maximum between 28 and 35 days of treatment.

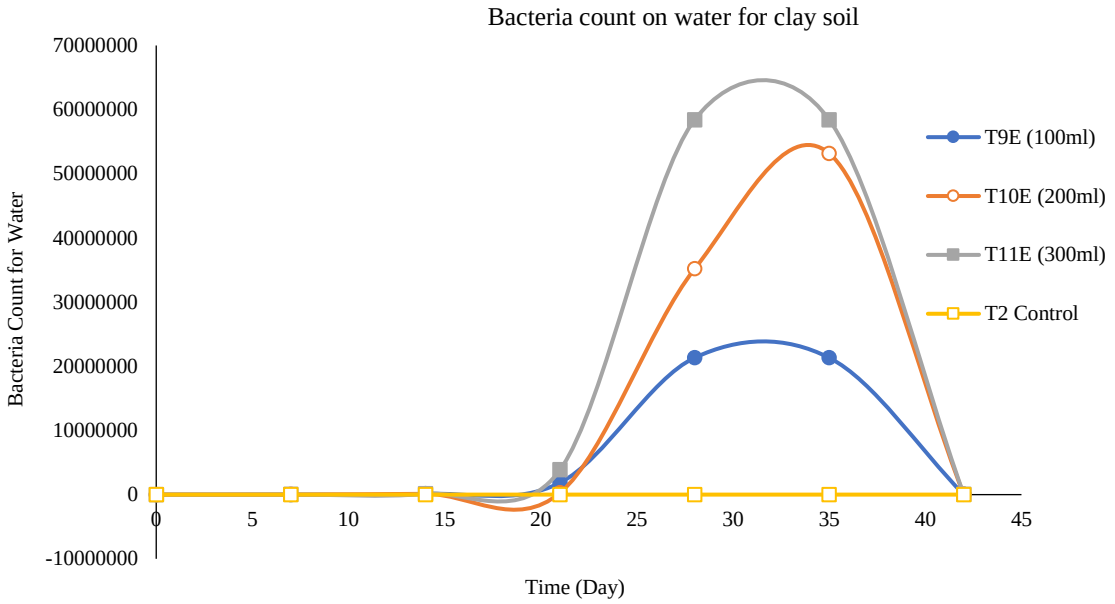

Figure 3. Effect of bacteria count on crude oil degradation in clay soil from water biostimulant.

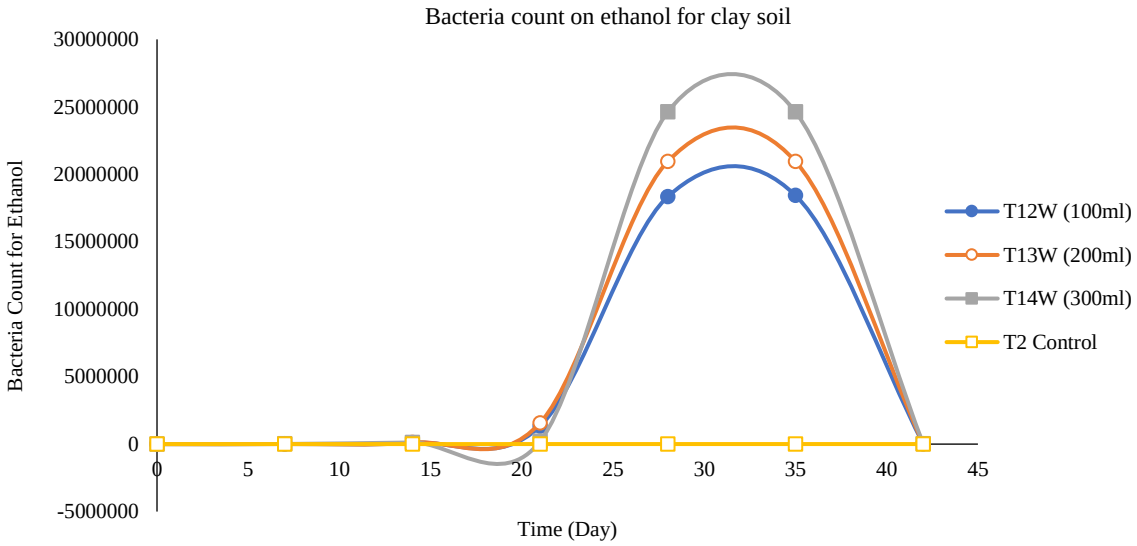

Figure 4. Effect of bacteria count on crude oil degradation in clay soil from Ethanol Biostimulant.

The increase in time of remediation had significant influence on the degradation level of hydrocarbon content in the sample. On the 42 days of the treatment, there was a decline in bacteria growth rate of 121.47×10^2 cfu/ml that increased total organic carbon, phosphorus, and nitrogen cycling in low contaminated loamy soil. Additional 200 ml biostimulant of ethanol and water increased the bacteria growth of the treated soil in 7 days from 117.27×10^3 to 213.31×10^5 cfu/ml and 300 ml biostimulant of water from 131.90×10^3 to 584.16×10^6 cfu/ml on 35 days, then toward 42 days of treatment recorded 108.10×10^3 cfu/ml which suggests that degradation only occurred from 7 to 35 days of treatment. The biostimulant provided the needed nutrients for bacteria to thrive in hydrocarbon environment. Similar observation occurred in Figure 4 with different mix proportion of nutrients with peak of bacteria population on 35 days of treatment of 253.96×10^6 cfu/ml using 300 ml. As established, the process of microbial degradation of petroleum includes steps such as contact time with hydrocarbons that use them as source of energy. The earlier stage of treatment with increase in nutrients enhanced rapid bacteria growth that modulates the hydrocarbon content of crude oil in soil.

CONCLUSION

The findings from the research were used in drawing out the conclusion as stated below:

- Investigation on bacteria counts recorded an increase as the amount of biostimulant increases; and towards the 42 days of treatment, there was a decline due to paucity of hydrocarbon in the media which the microbes feed on.
- The bacteria counts were more at the progressive phase.
- The degradation of the TPH increases with increase in bacteria counts.
- The bacteria count was more on ethanol extracts compared to water extract.

REFERENCES

1. Dowling DN, Doty SL. Improving phytoremediation through biotechnology. Curr Opin Biotechnol. 2009; 20(2): 204–206.
2. Dures OVA, Hornı KM, Pipi S, Gubis OVA. Rhizofifiltration potential of for cadmium and zinc removal from contaminated wastewater. Chem Pap. 2014; 68(11): 1452–1462.
3. Fodelianakis S. Allochthonous bioaugmentation in ex situ treatment of crude oil-polluted sediments in the presence of an effective degrading indigenous microbiome. J Hazard Mater. 2015; 287: 78–86.
4. Folch A, Vilaplana M, Amado L, Vicent R, Caminal G. Fungal permeable reactive barrier to remediate groundwater in an artificial aquifer. J Hazard Mater. 2013; 262: 554–560.
5. Frascari D, Zanaroli G, Danko AS. In situ aerobic metabolism of chlorinated solvents: a review. J Hazard Mater. 2015; 283: 382–399.
6. Frutos FJG, Perez R, Escolano O, Rubio A, Gimeno A, Fernandez MD, Carbonell G, Perucha C, Laguna J. Remediation trials for hydrocarbon-contaminated sludge from a soil washing process: evaluation of bioremediation technologies. J Hazard Mater. 2012; 199–200: 262–271.
7. Frutos F, Escolano O, Garcıa S, Mar Babın M, Fernandez MD. Bioventing remediation and ecotoxicity evaluation of phenanthrene-contaminated soil. J Hazard Mater. 2010; 183(1–3): 806–813.
8. Fuller ME, Kruczek J, Schuster RL, Sheehan PL, Arienti PM. Bioslurry treatment for soils contaminated with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). J Hazard Mater. 2003; 100(1–3): 245–257.
9. Galdames A, Mendoza A, Orueta M, de Soto García IS, Sánchez M, Virto I, Vilas JL. Development of New Remediation Technologies for Contaminated Soils Based on the Application of Zero-Valent Iron Nanoparticles and Bioremediation with Compost. Resource-Efficient Technologies. 2017; 3(2): 166–176.
10. Garcı´a Y, Ruiz C, Mena E, Rodrigo MA. Removal of nitrates from spiked clay soils by coupling Electro-kinetic and permeable reactive barrier technologies. J Chem Technol Biotechnol. 2014; 90(9): 1719–1726.
11. Jorgensen KS, Puutstinen J, Suortt AM. Bioremediation of Petroleum Hydrocarbon-Contaminated Soil by Composting in Biopiles. Environ Pollut. 2000; 107(2): 245–254.
12. Obiakalaije UM, Makinde OA, Amakoromo ER. Bioremediation of Crude Oil Polluted Soil Using Animal Waste. Int J Environ Bioremediat Biodegrad. 2015; 3(3): 79–85.
13. Ofoegbu RU, Momoh YOL, Nwoaogazie IL. Bioremediation of Crude Oil Contaminated Soil Using Organic and Inorganic Fertilizers. J Pet Environ Biotechnol. 2015; 6(1): 198–203.
14. Ogu GI, Odo BB. Crude oil bioremediation efficiency of indigenous soil fungal community spiked with cassava peels in Niger delta region, Nigeria. The International Journal of Science & Technoledge. 2015; 3(12): 19–26.
15. Ojewumi ME, Anenih EV, Taiwo OS, Adekeye BT, Awolu OO, Ojewumi EO. A Bioremediation Study of Raw and Treated Crude Petroleum Oil Polluted Soil with Aspergillus Niger and Pseudomonas aeruginosa. J Ecol Eng. 2018; 19(2): 226–235.
16. Ukpaka CP, Ugiri AC. Biodegradation kinetics of petroleum hydrocarbon in soil environment using Mangnifera indica seed biomass: A mathematical approach. Chem Int. 2022; 8(2): 77–88.

17. Uzoije AP, Onunkwo AN, Egwuonwu R. Crude oil sorption onto groundnut shell activated carbon: kinetic and isotherm studies. Res J Environ Earth Sci. 2011; 3(5): 555–563.
18. Xu P, Ma W, Han H, Jia S, Hou B. Isolation of a naphthalene-degrading strain from activated sludge and bioaugmentation with it in a MBR treating coal gasification wastewater. Bull Environ Contam Toxicol. 2015 Mar;94(3):358–64.
19. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao X, Li F, Li H, Yu H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. Front Microbiol. 2018; 9: 2885. Retrieved from doi: 10.3389/fmicb.2018.02885.
20. Yadav BK, Siebel MA, Bruggen JJA. Rhizofifiltration of a heavy metal (Lead) containing wastewater using the wetland plant Carex pendula. Clean Soil Air Water. 2011; 39(5): 467–474.

