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Microbial Remediation of Crude Oil Contaminated Soil using Animal Waste (Chicken Droppings and Cow Dung) with Degrading Potentials

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Abstract

The microbiological and physiological analysis of crude oil contaminated soil amended with animal wastes (Chicken droppings and Cow dung) were investigated using standard cultural techniques and different concentrations of nutrients amendments (10%, 30% and 50%). The present study was aimed to isolate and identify microorganisms from animal wastes with the potential of degrading/utilization of crude oil as a sole carbon source. Result obtained revealed increased soil organic matter and reduced soil nitrate and phosphorus, thus imposing a condition that impaired oil degradation in the soil. Treatment of the soil with 50% of cow dung resulted in the highest oil degradation rate of 85.78%. The bacterial isolates with crude oil degrading potentials were *Psuedomonas sp*. (88%), *Micrococcus sp*. (75%) *Bacillus sp*. (100%) *Klebsiella* and *Seratia sp*. Had the lowest percentage occurrence of 35% and 25% respectively. Animal waste can therefore be an option for crude oil pollution remediation.

Keywords: Hydrocarbon; Biodegradation; Animal waste; Hydrocarbonoclastic; Contaminated Soil

Introduction

Hydrocarbons are regarded as the most primary energy and fuel resources in the world. Accidental discharge/spillages occur during exploration and distribution due to acute accidents through human activities. Hence hydrocarbon is the trending pollutant of soil and water [1,2].

Crude oil is known to be a various chemical composition mixed together to form a chemical compound. Petroleum hydrocarbons are the major constituents of crude oil is petroleum hydrocarbon (50-98%) and 20-50% of alkenes as oil, depending on the source of the oil. Microorganisms such as bacteria, fungi, yeast and microalgae can degrade petroleum hydrocarbons. However, various researches have shown that bacteria play an imperative role in hydrocarbon degradation. Microorganisms possess metabolic machinery to utilize petroleum products as a carbon and energy source. Several factors such as molecular composition of the hydrocarbons, type of microbial population, and optimal environmental conditions to stimulate the bioavailability of the contaminants to microorganisms [3].

The process of bioremediation, defined as the application of microbial seeders to eliminate or cleanup pollutants due to their degradation potential is the trending measure for the removal of many environmental pollutants such as petroleum industry products [4]. Biosurfactants which are surface active agents is one of the most important characteristics of their exudative for the emulsification of hydrocarbon [1,2].

There are quite a few reports of isolation of petroleum hydrocarbon degrading bacteria [5]. conducted study and examined the effectiveness of *Psuedomonas sp*. culture that could degrade high percent of aliphatic compounds. Hydrocarbon degradation through the use of microbial seeders has been an outstanding and ultimate natural approach to detoxify/cleanup of petroleum hydrocarbon pollutants from the environment.

In the present study, microorganisms were isolated from animal waste were introduced into crude oil polluted soil. The efficacy of these microorganisms on crude oil was monitored. The aim of the present study was to isolate and identify microorganisms from animal waste with potentials of degrading crude oil. Hence, these organisms can be used as microbial seeders to solve crude oil pollution in contaminated sites.

Materials and Methods

Sample collection

Qua Iboe crude oil obtained from ExxonMobil Producing Nigeria Unlimited, Qua Ibeno Terminal (QIT), Eket, Akwa Ibom State, Nigeria was used in this experiment and stored at ambient temperature in a dark place throughout the experimental period. The soil samples from farmland was collected using soil sampler to a depth of 20 cm, stored in polythene bags and transported to the laboratory. The soil sample was air dried, sieved through 2 mm mesh and stored in polythene bags at room temperature (28 ± 2°C). Crude oil was sterilized in the autoclave at 121°C for 15 min before use to eliminate the autochthonous hydrocarbon degraders.

Medium used for screening and isolation of crude oil degrader

Liquid mineral salt medium with crude oil as a sole carbon source was used to maintain bacterial strains. Isolated of bacterial isolates was done by using an enrichment culture with single composition of

mineral salt and 20 g Agar-agar to plate-out the isolates on culture plates according to Lui et al., [6] Culture media used in isolation of bacteria was Nutrient agar (Oxoid Ltd; England) and the media were autoclave at 121°C for 15 min under 15 p.s.i pressure. The composition of the mineral salt medium was; 0.2 g of $MgSO_4$.$7H_2O$, 0.02 g of $CaCl_2$, 1 g of KH_2PO_4, 1 g of K_2HPO_4, 1 g of NH_4NO_3, 2 drops of $FeCl_3$ 60%. The pH was adjusted to 7.0-7.8. An enrichment culture and a single colony isolation technique were employed in during bacteria isolation.

Incorporation of amended materials into soil samples

Two thousand (2000) grams of soil was moistened and kept at room temperature (28 ± 2°C) in the microbiology laboratory for one week in duplicate. The soil sample was contaminated with crude oil at the ratio of 1:5 and kept for two (2) weeks. A basal dressing of poultry droppings and cow dung was applied at the ratio of 10%, 30% and 50%. The experimental sample was set up as shown in Table 1. Both amended soil and control was incubated at room temperature (28 ± 2°C) and observed after every two weeks for 12 weeks after pollution and the effect of the amended sample studied. Thereafter, oil content was estimated. Change in oil content in the treatment was calculated relative to the oil content in the population and unamended control. Pure culture was prepared by plating out of the enrichment broth on nutrient agar. The incubation period to ascertain the degradation potentials of bacterial isolates in animal waste was (12) weeks.

S/NO	CONDITION	DESCRIBTION OF CONDITON
PC1	Chicken dropping added @10%	100 g of pollution soil+10 g of chicken droppings
PC2	Chicken dropping added @30%	100 g of pollution soil+30 g of chicken droppings
PC3	Chicken added 10%	100 g of pollution soil+50 g of chicken droppings
PC4	Cow dung added @10%	100 g of pollution soil+10 g of cow droppings
PC5	Cow dung added @30%	100 g of pollution soil+30 g of cow droppings
PC6	Cow dung added @50%	100 g of pollution soil+50 g of cow Droppings

Table 1: Test Condition and Samples Treatment.

Results and Discussion

physico-chemical analysis of samples

The result of the physico-chemical parameter of polluted soil monitored after two weeks showed increase in the soil pH from (5.67-5.99) and decrease in available phosphorus from (29.99-3.00) and total nitrogen from (0.27-0.06), exchangeable Ca from (2.82-2.46), Mg (1.20-0.98), Na (0.05-0.04) and K (0.08-0.06) in the crude oil polluted soil when compared to values obtained from unpolluted soil which served as control. The clay (15.02) and silt (19.75) particles of the polluted soil were higher than those of the unpolluted soil clay (14.50) and silt (19.10) when compared (Table 2). The physico-chemical analysis of polluted soil amended with animal wastes from baseline (Day 1) to week 12. The results revealed an increase in the electrical conductivity, total Nitrogen, Ca, Na and K when compared to the values obtained from unamended polluted soil.

The experimental design for soil treated with crude oil and amended animal wastes are presented in Table 1. At the end of the incubation period, 85.78% and 56.81%, of crude oil was degraded in soil treated with animal waste (Chicken droppings and Cow dung) respectively. The enhanced oil degradation observed with cow dung and chicken droppings indicated that the soil amendments induced enhanced crude oil degradation. Maximum oil reduction of 320.74 ppm, reductive to the unamended control was observed from treated sample with cow dung.

Observably, the total mean heterotrophic count of bacteria in the polluted soil amended with wastes ranged from 1.56-3.31 × 10^8 cfu/g. the total mean count of hydrocarbon degrading bacteria ranged from 1.14-1.66 × 10^4 cfu/g. The total heterotrophic count of unpolluted soil sample was 4.8 × 10^8 cfu/g and the values from heterotrophic count from soil sample were comparatively higher than that of hydrocarbon degraders (0.6 × 10^4 cfu/g).

Conversely, the highest mean count of 1.54 × 10^4 cfu/g was recorded by cow dung sample for petroleum hydrocarbon utilization and 1.69 × 10^5 cfu/g for total for total heterotrophic fungi mean count. There was increase in the petroleum hydrocarbon utilizers from day 1 to week 6 and decrease sequentially from week 8 to week 12. The result revealed the frequent occurrence of the organisms during the analysis. It was revealed that *Bacillus* had 100% occurrence and hydrocarbonoclastic potential followed by *Psuedomonas and Mirococcus* with 85% occurrence. *Klebsiella and Seratia sp.* Had the lowest percentage occurrence of 35% and 25% respectively.

The use of animal waste (Chicken droppings and Cow dung) is an innovative approach in bioremediation of crude oil due to the natural abundance of nutrients and stable pH value for microbial proliferation to facilitate the biological remediation process. Therefore, to maintain a fertile soil (farmland) polluted with crude oil it is recommended to be with adequate supply of Nitrogen and Phosphorus necessary for plant growth, animal waste such as chicken droppings and cow dung are essential to serve as nutrients and enhance the proliferation of organisms that are capable of degrading and utilizing crude oil as their source of carbon and energy.

Chemical analysis	Soil Properties Before crude oil Pollution	After oil Pollution
pH		
Ec (ds/m)	5.67	5.99
Organic Matter%	0.04	0.048

Total Nitrogen%	2.29	10.94
Available phosphorus (mg/kg)	0.27	0.06
Exchangeable Ca (Cmol/Kg)	29.99	3.00
Exchangeable Mg (Cmol/Kg)	2.82	2.46
Exchangeable Na (Cmol/Kg)	1.20	0.98
Exchangeable k	0.05	0.04
Exchange acidity	0.08	0.06
Effective Cation	1.03	2.66
Exchange Capacity (Cmol/kg)		
Base saturation	6.81	4.58
Particles size analysis	60.94	77.51
Sand%	66.40	66.40
Silt%	19.10	19.75
Clay%	14.50	15.02
Textural Class	**Sandy loam soil**	**Sandy loam soil**
Microbiological Analysis Bacterial Populations		
Total Heterotrophs (10^8 cfu/g)	4.8	3.2
Petroleum Hydrocarbon utilizers(10^4 cfu/g)	0.6	1.8

Table 2: Soil Properties Before Crude Oil Pollution And Two Weeks After Crude Oil Pollution.

Conclusion

Petroleum hydrocarbon cleanup on the lithosphere is a world problem. A better knowledge and mechanism essential for biodegradation has high ecological significance that is dependent on the alternative source of allochthonous hydrocarbon degrading bacteria from farm waste such as chicken droppings and cow dung.

This work revealed that animal wastes could be used in bioremediation of crude oil pollute soil for enhanced remediation of polluted agricultural soil. These bacterial isolates obtained from animal waste (Chicken droppings and Cow dung) possess biodegradation potentials and expressed competence in utilizing crude oil as a carbon source. The bacterial isolates with crude oil degrading potentials obtained were *Psuedomonas sp.* (88%), *Microccocus sp.* (75%), *Bacillus sp.* (100%) *Klebsiella* and *Seratia sp.* Had the lowest percentage occurrence of 35% and 25% respectively.

The result revealed that these bacterial isolates obtained from animal waste expressed abilities to utilize Qua Iboe crude oil as a sole carbon source and can be very effective when applied as microbial seeders to serve as bioremediation approach on crude oil contaminated environment. Applying this scientific research, animal waste (Chicken droppings and Cow dung) are recommended for the elimination of crude oil pollutant as bioremediation approach on polluted soil and rejuvenation/maintenance of natural land value for farming purposes.

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