Comparison of the Effect of Fresh and Dry Lemon Grass (*Cymbopogon citratus*) Granules on Biodegradation of Petroleum Hydrocarbons

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Abstract: **This work compared the result of the effect of lemongrass granules as a local raw material in the treatment of crude oil polluted soils when it is in a fresh or dry condition. The comparison of the usage in fresh or dry state is aimed at identifying the most effective among the two conditions that will give the best result in biodegradation of petroleum hydrocarbons. The soil samples were obtained with hand soil auger and analyzed in the laboratory using standard methods. Soil sample C represented the soil treated with fresh lemongrass while soil sample E was treated with dry lemongrass. Poultry droppings were added to them as nutrients while the experiment was monitored for 28days. The determined parameters were total petroleum hydrocarbon, pH, total nitrogen and phosphorous and hydrocarbon utilizing bacteria. The results show that the soils samples were Clay soils and total petroleum hydrocarbon decreased by 77% and 24 % for soil sample C and E respectively. The soil pH values were 6.51 and 6.10 for soil sample C and E. Also total nitrogen was 0.11% and 0.355% for soil sample C and E. The concentration of phosphorous in soil sample C and E were 0.24 and 1.26% respectively. The hydrocarbon utilizing bacteria count increased from $3.65x10^3$ cfu/g to $5.41x10^3$ cfu/g in soil sample C but decreased to $2.10x10^3$ cfu/g in soil sample E. Comparison of the two process showed a significant decrease in concentration of total petroleum hydrocarbon using fresh lemongrass granules to dry lemongrass granules. This implies that the use of fresh lemon grass is more effective compared to that of dry lemon grass granules. This is because the use of fresh lemongrass granules enhanced rapid growth of microorganism, therefore a significant result on biodegradation of petroleum hydrocarbon was achieved. This confirmed the use of fresh lemongrass granules as a better method compared to dry lemon grass granules in the treatment of soils contaminated with petroleum hydrocarbons.**

Keywords: **Biodegradation, Dry lemongrass granules, Fresh lemongrass granules and Soils samples**

I. INTRODUCTION

Bioremediation is a process that had to do with the application of microorganisms to break down or transform contaminants to non or less harmful states (Jelena *et al*., 2012). It involves transformation of the physicochemical characteristics of the contaminants with the use of microorganism. The microorganisms responsible in the process carried out their activities through the use of enzymatic pathways such as biocatalysts which facilitate biochemical process that breakdown the pollutants. Many factors are responsible for an effective and efficient bioremediation process. Such factors could be the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms (Abatenh *et al.,* 2017). Bioremediation is effective in an environment with sufficient oxygen and nutrient. It is only in rare cases that biodegradation occurs in environment with insufficient oxygen (Abatenh *et al.,* 2017). Biodegrading bacteria require sufficient oxygen for breakdown of petroleum hydrocarbons (Atlas and Philips, 2005). Also, one of the major factors for effective bioremediation of petroleum hydrocarbon is the availability of nutrients. The most essential nutrients are nitrogen and phosphorous. The application of nitrogen and phosphorous increases the proliferation of biodegrading bacteria, resulting in an increase in degradation rates (Rosenberg *et al.,* 1996). This work looked at the best condition lemongrass granules can be applied on biodegradation of petroleum hydrocarbons to obtain its effectiveness. The results obtained from the use of fresh or dry lemongrass granules were compared to ascertain the most effective process. Lemongrass (*Cymbopogon citratus*) is plant, mostly cultivated in warm tropical and subtropical regions (Naik *et al*., 2010). It has about 1% to 2% of essential oil when it is on dry condition. The chemical component differs based on its function of genetic diversity, habitat, and agronomic treatment of the culture (Carlson *et al*., 2001). The objective of this work is to compare the outcome obtained using fresh and dry lemongrass granules on biodegradation of soils contaminated with petroleum hydrocarbon. The comparison will assist to determine the most effective process using fresh or dry lemongrass granule in bioremediation processes.

II. MATERIALS AND METHODS

Soil Sampling

The soil samples were collected using hand soil auger from Oshiobele Community in Ahoada West Local Government Area of Rivers State. The collected soil samples were bulked together and put into well labeled glass bottles and sealed with aluminum foil, and used for Total Petroleum Hydrocarbon

(TPH) analysis (Umeda &Ollor, 2019), while the poultry droppings and dry lemon grass were collected from the Rivers State University Farm (Umeda *et al.,* 2021).

Experimental Procedure

Dry lemon grass was obtained by drying 30kg of fresh lemongrass at room temperature for 14 days. Twenty kilogram (20 kg) each of fresh and dry lemon grass were separately converted into granules using a grinding machine. This is to allow easy absorption of the lemongrass by the petroleum contaminated soils by reducing their surface areas (Umeda *et al*., 2021). One hundred and fifty kilogram (150kg) of soil samples were weighed into a bowel and contaminated with 15000mls (15litre) of Bonny light crude oil by using standard pollution volume of 100mls of crude oil to 1kg of soil. The mixture was properly mixed to ensure uniform concentration of the crude oil in the soil samples and left for three (3) days to settle without any disturbance. Thereafter, fifty (50) kilogram each of the contaminated soils were obtained and transferred into three different bowels labeled A, C and E. The treatment of the soils commenced after three (3) days with mixing of 1000g of fresh lemon grass granules with soils sample C while another1000g dry lemongrass granules were added to soil sample E. Furthermore, four hundred and fifty gram (450g) of poultry droppings each were added separately to soil samples C and E as nutrients. Soil samples A had petroleum contaminated soils without any treatment and it represents the control sample for the experiment. One hundred and fifty (150mls) milliliters of water was sprinkled on the soil samples in A, C and E every two days to enhance the moisture content of the contaminated soils. The process was monitored for 28days. Soil samples were collected from soil sample A, C and E every seven (7) days for analysis in the laboratory (Umeda *et al*., 2021).

Determination of Physicochemical Properties

The soil samples were analyzed for Particle Size Distribution (PSD) and classification, pH, total petroleum hydrocarbon, total nitrogen, and phosphorous. The physicochemical properties were determined using standard methods adopted from relevant literatures**.** Soil type classification and particle size analyses were carried out before contamination of the soil with crude oil by hydrometer method using sodium hexametaphosphate as the dispersing agent (Ayotamuno et al., 2011). The soil structural classification was obtained, using the United State Department of Agriculture (USDA, 1987) soil textural classification scheme using TAL®for Windows software., The pH levels of the soil samples were determined in the laboratory using Hanna HI 2211 pH/ORP meter according to ASTM (1999) method D4972. Total Petroleum Hydrocarbon was analyzed by using Gas Chromatograph-Flame Ionization Detector (GC-FID) Model, HP 5890 Series II, U.S.A., after extraction of hydrocarbon content by applying ASTM (1999) method D3921(Umeda et al., 2019). Total nitrogen was determined by using APHA (1998) method, 4500-NO3 B while phosphorous was analyzed by using APHA (1998) method, 4500-$PO43^{-}$. In cultivation of total heterotrophic bacteria, prepared nutrient agar culture plates were made according to the manufacturer's specification (HIMEDIA) M001-500G, HIMEDIA Laboratories Pvt. LTD Number-400086, India). The culture plates were dried and 0.1ml of the 10^1 diluted soil sample was placed on it using sterile pipette and spread using a sterile glass rod spreader to dryness on the plate. This was incubated in an incubator at 37^{O}C for 24hours and the counting of the bacteria was made on the plate after the bacteria have shown growth. The bacteria that couldn't grow at the end of 24 hours were further allowed incubated in the incubator for another 24hours. (Barrow & Feltham, 2003). Also, the Vapour phase technique was used to grow and identify the hydrocarbon utilizing bacteria. The culture plates were prepared by using the Mineral salt agar without the carbon source (Okpokwasili & Odukoma, 1994). The plates were dried and 0.1ml of the 10^1diluted soil samples were placed on the dried plates. The samples were spread on the minerals salt agar plate using a sterile glass rod spreader to dryness. A crude oil (Bonny light) soaked on ninety (90mmø) millilitre diameter Whatman filter paper No.1 (Whatman International Ltd Maid store, England) was placed on the cover of the cultured plates and were incubated at room temperature for initial three(3days) with observation and extended to seven(7days) for extended observation. The hydrocarbon utilizing bacteria were counted during the periods and recorded accordingly. Further tests were carried out to identify the bacteria using Okpokwasili & Odokoma,1990 techniques. To identify the isolated bacteria, pure cultures of the isolates were prepared by aseptically streaking representative colonies of the different cultures, which appeared on the culture plates, onto dried nutrient agar plates and incubated at 37^{O}C for 24 hours. The nutrient agar plates were stored in a refrigerator and this served as pure stock culture for subsequent characterization and identification tests. Standard characterization tests (such as Gram staining, motility, oxidase test, and catalase and other tests) were performed. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics (Barrow & Feltham, 2003). The Degree of degradation of hydrocarbon was obtained using Equation (1) below.

$$\% D = \frac{TPHi - TPHf}{TPHi} \times 100 \qquad (1)$$

where TPH_i and TPH_f represent the initial and final concentrations of Total Petroleum Hydrocarbon (Umeda & Ollor, 2019).

III. RESULTS AND DISCUSSION

Particle Size Distribution (PSD) and soil classification revealed that the soil was Clay soil with 9.50% Sand, 15.30% Silt, 75.20% Clay, 1.432g/cm Bulk density and 0.461 Porosity.

Variation of Total Petroleum Hydrocarbon with Time

Figure 1 shows the comparison of the effect of fresh and dry lemon grass granules on Total Petroleum Hydrocarbon for soils sample in A, C and E. The graph shows that there was appreciable decrease in concentration of Total Petroleum Hydrocarbon in soil sample C compared to soils sample A and D.

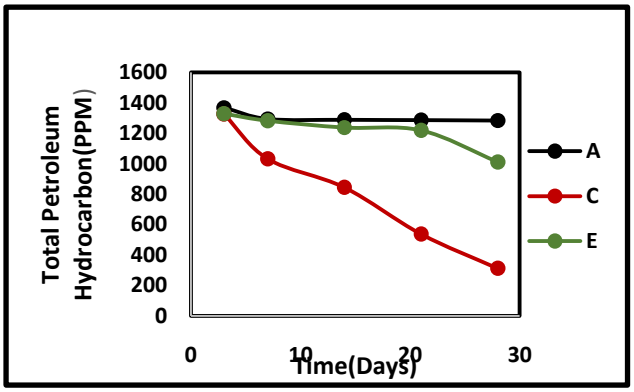

Figure 1: Comparison of the Effect of Fresh and Dry Lemon Grass Granules on Total Petroleum Hydrocarbon.

The result indicated that the use of fresh lemon grass granules decreased the concentration of total petroleum hydrocarbon by 77% while dry lemon grass granules decreased it by 24%. This implied that fresh lemongrass granules are more effective in biodegradation of petroleum hydrocarbons than the use of dry lemon grass granules. The effectiveness of fresh lemongrass granules noticed on biodegradation of the hydrocarbon may be as a result of its ability to promote the growth of microorganisms that depend on the hydrocarbon for energy.

Variation of pH with Time

Figure 2, shows the comparison of the effect of fresh and dry lemon grass granules on pH for soil sample A, C and E. At 28 days, the level of acidity in the soils were 4.52, 6.51 and 6.10 respectively. This implied that there was an increase in the acidity levels in soils sample A while soils sample C and E had significant decrease. The decrease was more appreciable in soil sample C.

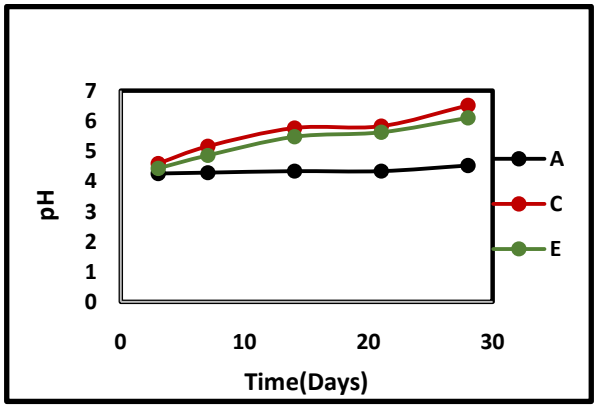

Figure 2: Comparison of the Effect of Fresh and Dry Lemon Grass Granules on pH.

Also, the decrease in acidity level of soil sample C and E may be due to the combination of poultry droppings as nutrient with fresh and dry lemongrass granules which reduced the acidity level of the petroleum polluted soils thereby provided biochemical reactions that did not increase the acidity of the soil during the biodegradation process.

Variation of Total Nitrogen with Time

Figure 3, shows the comparison of the effect of fresh and dry lemon grass granules on total nitrogen in biodegradation of petroleum hydrocarbons. The results obtained were 0.4%, 0.11% and 0.355% for soil sample A, C and E respectively. It indicates that soils sample A and E had slight decrease in concentration of total nitrogen while soil sample C showed a significant decrease.

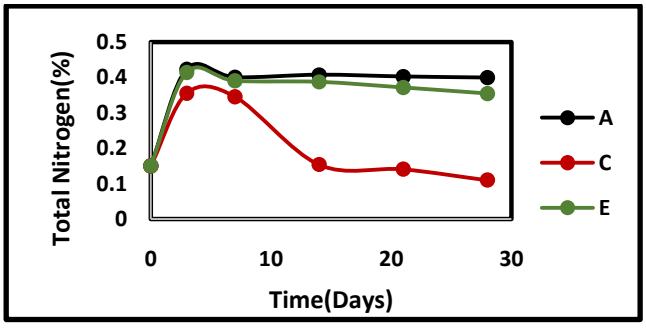

Figure 3: Comparison of the Effect of Fresh and Dry Lemon Grass Granules on Total Nitrogen.

The appreciable decrease noticed in soil sample C indicates that fresh lemongrass supports the growth of microorganism that utilized nitrate as nutrient for energy while the slight decrease of total nitrogen in soil sample E revealed that the application of dry lemongrass granules in biodegradation of petroleum contaminated soils is less effective.

Variation of Phosphorous with Time

Figure 4 shows the comparison of the effect of fresh and dry lemon grass granules on phosphorous for soils sample A, C and E. The results obtained were 1.52%, 0.24% and 1.26% respectively. The results show that there was a significant decrease in concentration of phosphorous in soils sample C compared to soil sample A and E.

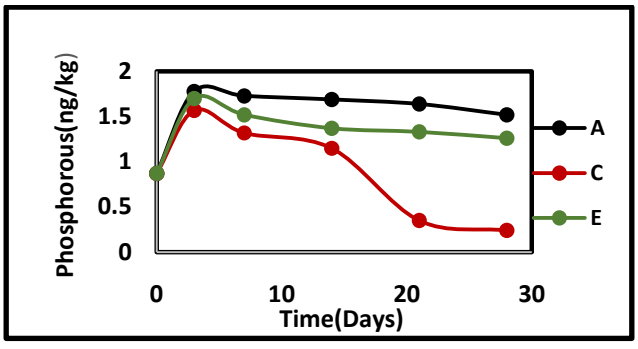

Figure 4: Comparison of the Effect of Fresh and Dry Lemon Grass Granules on Phosphorous.

The rapid decrease in concentration of phosphate in soil sample C may be due to increase in population of microorganism aroused from the use of fresh lemongrass in combination of poultry droppings which provided room for higher demand for phosphate as nutrient for energy in the bioremediation process.

Variation of Hydrocarbon Utilizing Bacteria with Time

Figure 5, shows the comparison of the effect of fresh and dry lemon grass granules on hydrocarbon utilizing bacteria for soils sample A, C and E. The experimental results obtained were $1.9x10^2$cfu/g, $5.41x10^3$cfu/g and 2.10^3cfu/g respectively. In line with the results, the graph revealed that, there was a decrease in population of hydrocarbon utilizing bacteria in soils sample A and E while an increase was noticed in soil sample C.

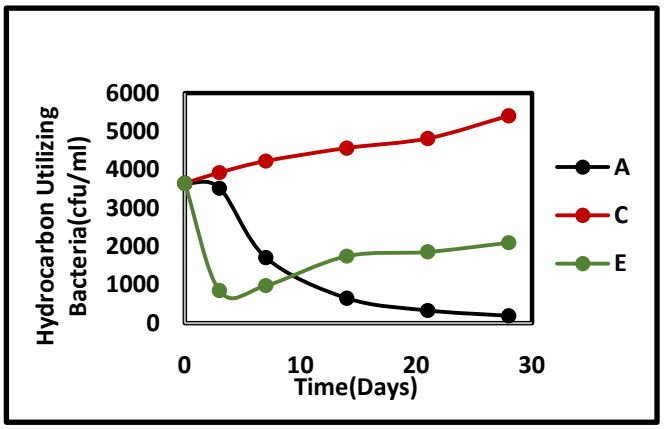

Figure 5: Comparison of the Effect of Fresh and Dry Lemon Grass Granules on Hydrocarbon Utilizing Bacteria.

The results obtained in soil sample E indicates that the combination of dry lemon grass with poultry droppings did not support the growth of microorganism which resulted in poor performance to reduce the concentration of petroleum hydrocarbons unlike the combination of fresh lemongrass and poultry dropping in soil sample C which enhanced microbial growth, hence, provided a speedy and efficient process of biodegradation of petroleum hydrocarbon in the soil.

IV. CONCLUSION

The results obtained from the experiment indicate that the concentration of total petroleum hydrocarbon decreased by 77% and 24% when lemongrass granules were applied in fresh and dry state respectively using poultry droppings as nutrient. This implies that the use of lemon grass granules in fresh condition or state is more effective compared to its usage in dry condition in biodegradation of petroleum hydrocarbons in the soil. This is because the usage in fresh condition or state enhanced rapid growth of microorganism that biodegraded the petroleum hydrocarbons, therefore a significant result was achieved.

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