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Bioremediation of Crude Oil-Contaminated Soil using Compost as Bio-Stimulant

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ABSTRACT: This study was conducted to investigate the usability of compost as a stimulant for the remediation of crude oil-contaminated soil. 2kg of porous sandy soil was measured into six different equal-sized cells and contaminated with 250 ml of sample crude oil. Masses of solid waste comprising of compost and sawdust were measured in varying amounts of 900g, 750g, 600g, 450g, 300g, and a control sample that had no compost and sawdust. The set-up was left to stand for eight weeks and the residual hydrocarbon content (RHC), pH, and total microbial count (TMC)of these samples were monitored and recorded. The percentage of hydrocarbon degradation that occurred in the treatment cells were; 33.11, 32.2, 31.2, 29.46 and 28.05 respectively, for the individual cells in the order above, while the control had a degradation rate of (0.44%) of the total hydrocarbon content after 8 weeks. pH values varied slightly within the ranges of 6.5-7.5. The TMC average values were21, 188, 17,125, 15,000, 14,250, 13,125 and 0.1, with the highest count occurring in the sample having 900g of solid waste; followed by the other samples having lesser quantities of solid waste with the least value in the control. The resultant descent in TMC across each of the samples was due to the distribution of waste material in the samples, and the pH of the resulting mixtures remained at optimum, thereby favouring microbial growth across the samples.

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Petroleum-based products are the major sources of energy for industry and daily life and as the world's dependence on crude oil and its derivatives increases, so does the level of exploration. This has created the conditions for the potential distribution of large amounts of toxins associated with crude oil into the environment (Obahiagbon *et al*., 2014). Decontamination of sites by biological means has received significant attention.

Specifically, bioremediation has been identified as a sustainable and suitable option for the decontamination of crude oil-polluted soil, it has a relatively low cost of operation, low technology requirements, it is easily implanted and the pollutants are degraded into less toxic forms in a relatively short time (Erdogan and Karaca 2011; Otokunefor and Obiukwu, 2010). It involves the use of microorganisms with the capacity to degrade these hydrocarbons and mineralize them into simpler and less toxic forms such as CO_2 and H_2O (Amenaghawon *et al*., 2013). Bioremediation, as a cleanup method, is typically implemented through either of three strategies namely; natural attenuation, biostimulation, or bioaugmentation (Yu *et al*., 2005). Natural attenuation refers to the combination of natural processes that occur without human involvement, to decrease or "attenuate" contaminant concentrations and toxicity in land or wastewater, and thereby reduce the hazards posed by the contaminants. The process of externally stimulating microbial growth and activity for the remediation of contaminants is referred to as biostimulation. The low population of indigenous microorganisms is one of the major limitations of bioremediation through natural attenuation.

In some instances the indigenous microorganisms do not even possess the natural metabolic activity necessary to degrade the hydrocarbon pollutants hence the need for a specialized consortium of microbes which is added to the remediation medium exogenously (El Fantroussi and Agathos, 2005). Since biological processes are often highly specific, important site factors required for successful bioremediation like; the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrient (organic waste or compost), must thus be maintained (Vidali., 2001).

In this study, compost was used to stimulate the growth and multiplication of oil-eating microbes (bacteria and fungi) for the remediation of soil polluted with crude oil.

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MATERIALS AND METHODS

*Sample Collection and Preparation:*The soil sample was collected from the surrounding of the department of Chemical Engineering, University of Benin. Crude oil was obtained from NPDC flow station, Ologbo, Edo state. The collected soil sample was air-dried and mechanically homogenized by removing pebbles, plastics and metals, and screened using a 2.8mm mesh size sieve. Moisture content and pH of the test samples were determined to know their initial conditions. The solid waste (compost) consisted of poultry droppings, which was obtained from the faculty of agriculture, University of Benin.

2kg of soil sample was measured into 6 different cells and contaminated with 250ml of crude oil each, and mixed thoroughly using a hand trowel. Varying masses of solid waste (poultry droppings and sawdust) which served as amendments/bio-stimulants were weighed in masses of 0.3kg, 0.45kg, 0.6kg, 0.75kg and 0.9kg respectively, and were added to each of the five cells which served as the test samples. One cell was labelled the control sample and contained only crude oil and 2kg soil.

The 6 cells (i.e. the test samples and the control sample) were allowed to stand for one week, to allow for the growth and adaptation of indigenous microbes. Finally, bioremediation indication parameters; pH, Residual hydrocarbon content (RHC), and Total microbial count (TMC) were monitored for a period of 8weeks.

Determination of pH: The pH of the samples was determined using the procedure reported by (Satsangee *et al*., 1990). 20g of soil sample was webighed into 100ml beaker, mixed with 20ml of distilled water and stirred thoroughly. The mixture was allowed to stand for 30 minutes and then the pH value was determined using an electric pH metre (Phs 25 Techmel, USA).The electrode system of the pH meter was first calibrated using a standard buffer solution after which the sample was thoroughly mixed using a stirrer and its pH measured and recorded.

*Determination of Residual Hydrocarbon Content (RHC):*The residual hydrocarbon content of the soil was determined using a method described by (Osuji and Nwoye 2007). A mixture of 5g of contaminated soil and 50 ml of n-hexane was vigorously shaken for 20 minutes. The mixture was then left to stand for 20 minutes, after which it was filtered with Whatmann filter paper (no 2). The RHC was calculated after reading the absorbance of the extract from the spectrophotometer at a wavelength of 425 nm.

*Determination of Total Microbial Count (TMC):*Dilution of the soil sample was prepared by washing soil with distilled water and then diluting using the diluent already prepared (obtain 10^{-1}, 10^{-3} and 10^{-6} dilutions) using the procedure reported by (Bassiri, 2011). The Colony counting chamber was assembled by applying the cover glass. Few drops of Methylene blue solution were added to the water sample and the dilution. With a standard loop, a loop-full of water sample (including the various dilutions) was placed on the ruled area of the counting chamber and then the chamber was allowed to stand for 5mins. It was then examined under a microscope using an mm lens (x 16 objective lens) to count the bacteria in 50-100 square selected at random so that the total number of bacteria is about 500. For each sample, a triplicate was obtained, counted and divided by the number of counts, then by the number of squares and the result multiplied by the dilution factor and a constant k. This gave the number of organisms in a millilitre of the given water sample.

RESULTS AND DISCUSSION

*Variation of pH with time:*The result obtained for the pH of samples of varying masses over a period of 8 weeks is given below:

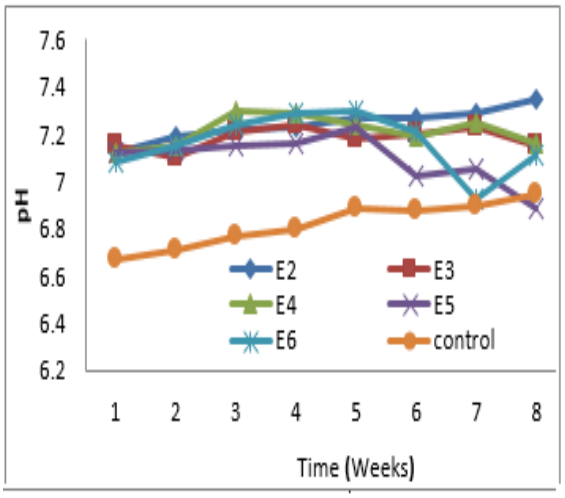

Fig 1: Variation of pH with time

It can be seen from the figure above that during the first week, all the samples had initial pH levels of 6.5-7.3. Subsequently, in the second week, there was a negligible increase in the pH of all the samples; this was as a result of the addition of organic material (compost) which increases the value of pH due to their slightly alkaline nature. Over time, there was a steady increase in pH, which may be due to the increase in microbial population and activities. In the 5th and 6th weeks, some samples began to experience lower pH levels, but these pH levels still provided optimum

conditions for biodegradation to occur, as they still fell within the optimum range of 6.5-7.5.

It can also be seen that the control (soil + crude oil) had the lowest value in pH in comparison with the samples containing solid waste (compost) throughout the eight weeks period, which was indicative of the fact that there was no addition of compost, hence, little or no microbial activity. Solid waste (compost) has helped in the remediation of the soil by increasing the pH to favour microbial activities (Golodyaev*et al*., 2009).

*Residual Hydrocarbon Content:*Figure 2 shows the residual hydrocarbon content throughout 8weeks. Bioremediation of petroleum and hydrocarbons in the soil is a complex process where quantitative and qualitative aspects depend on the nature and amount of hydrocarbon(s) present. It can be seen from Figure 2 that the RHC decreased with time for the various samples, in comparison with the control sample which had a negligible decrease in its RHC. This may have been because the control sample was void of compost; hence the microbial activities were minimal. After 8weeks of remediation, the RHC in each sample decreased quantitatively. This signifies that there was an improvement in the degree of remediation offered by the samples containing waste material (compost), over the control. It is also evident that the amount of amendment introduced affects the rate of bioremediation.

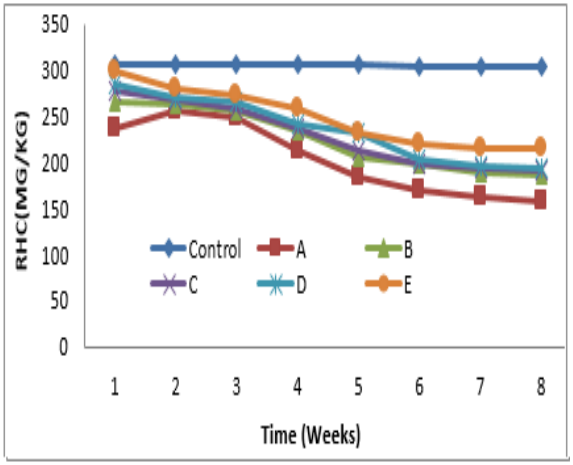

Fig 2: Variation of RHC with time

*Total Microbial Count (TMC):*Figure 3 shows the total microbial count (TMC) for 8weeks. It was observed that there was an increase in microbial count between week 1 and 2 for each of the samples, excluding the control which experienced little or no change. There was a corresponding increase in week 3, at this time the microbes had adapted to the toxic environment and began to multiply. It was also observed from the graph that there was a decrease in total microbial count in some of the samples in the fifth week. This may have been as a result of competition for food, space and nutrients amongst the microbes resulting in the death of some of microbes, hence a reduction in their population. Consequently, it can be said that microbial growth is dependent on the number of nutrients or food made available to the microbes which would affect the rate of biodegradation. Previous reports have it that a high number of certain oil-degrading microbes from the oil-polluted environment are evidence that these microbes are vigorous degraders of the pollutants in the environment (Okerentugba *et al*., 2003). Other reports associated the increase in proliferation to not only hydrocarbon degraders but additional populations that utilize the resultant products from hydrocarbon break-down (Atlas and Bartha, 1992; Okpokwasili *et al*., 1990).

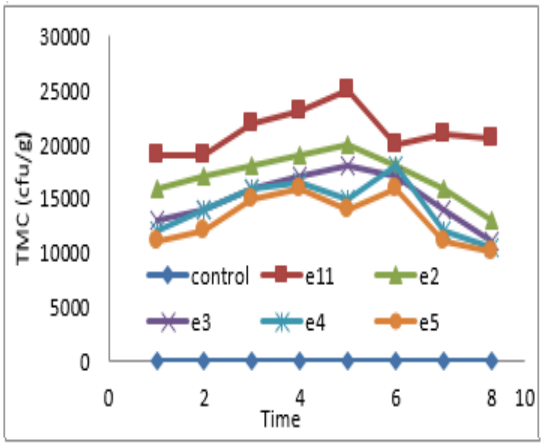

Fig 3: Variation of Total microbial count with time

*Conclusion:*This study was aimed at determining the efficiency of compost as a biostimulant in the remediation of crude oil contaminated soil. It can be concluded that an increase in the amount of biostimulant resulted in an increase in the total microbial count, which resulted in a proportionate increase in the remediation of crude oil-contaminated soil. It can also be concluded from this study that the amount of bio-stimulant applied in the remediation of crude oil contaminated soil does not have any significant effect on the pH of the soil during the process of remediation.

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