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Ebube P. Nwadiokwu
Department of Chemical Engineering, University of Benin, Nigeria.

Omotola I. Oladimeji
Department of Chemical and Petroleum Engineering, Afe Babalola University, Ado Ekiti, Nigeria.

Abdulkadir M. Zabi
Department of Energy Systems Engineering, Cyprus International University, Cyprus.

Omere S. Uyi
Department of Chemistry, University of Benin, Nigeria.

Antony A. Okakwu
Department of Chemistry, Kaduna State University, Nigeria.

Abdulfatai A. Akinola
Department of Chemical Engineering, University of Lagos, Nigeria.

Boluwatife S. Olubusoye
Department of Chemical Sciences, Olabisi Onabanjo University, Ago Iwoye, Nigeria.

Daniel A. Odogwu
Department of Chemistry, University of Benin, Nigeria.

Correspondence:
Boluwatife S. Olubusoye
Department of Chemical Sciences, Olabisi Onabanjo University, Ago Iwoye, Nigeria.

Bioremediation of Crude Oil Polluted Soil Using NPK 15:15:15 and Urea as a Biostimulant

Ebube P. Nwadiokwu, Omotola I. Oladimeji, Abdulkadir M. Zabi, Omere S. Uyi, Antony A. Okakwu, Abdulfatai A. Akinola, Boluwatife S. Olubusoye, Daniel A. Odogwu

Abstract
Crude oil pollution is harmful and dangerous to plants, animals, and humans hence the need to decontaminate sites polluted by crude oil. In this study, the bioremediation of crude oil using organic fertilizer (Urea), and inorganic fertilizer (NPK) was investigated. This was done using 4kg of soil per sample polluted with 1L of crude per kg of soil. Soil samples were contaminated with crude oil in four different cells (cells undergoing natural attenuation as the control, Urea alone, NPK alone, and NPK + Urea). These combinations were added as biostimulants to the indigenous microorganism present in the contaminated soil. After eight weeks, the pH of the samples was approximately found to be within the range of 6-8 in all the cells, satisfying that stipulated by FEPA. Also, the cell undergoing natural attenuation has the highest residual hydrocarbon content (RHC) and total microbial count (TMC) values of 3577.81 mg/kg (% degradation of 44.29) and 9 x 10^4 Cfu/g respectively after eight weeks. While the mixture of NPK and Urea has the lowest RHC and TMC values of 790.83 mg/kg (% degradation of 87.48) and 9 x 10^6 Cfu/g respectively. From these results, this study showed that though remediation occurred in all the cells, the cells stimulated by nutrients and their combination offered better remediation results.

Keywords: Biostimulant, Fertilizer, Bioremediation, Crude Oil, NPK, Urea.

1. Introduction
Nature can recycle and purify itself, but in recent years, the demand placed on the environment by huge amounts of anthropogenic pollution exceeds its capacity to recover. Conventional methods of dealing with oil spills include using dispersants or collecting the oil plume or through bioremediation. (Agunwamba, 2004). The emission rates of greenhouse gases produced from the use of fossil fuels pose a threat to the world climate and have led to continuous research on how to reduce it (Oladimeji et al., 2022). Oil pollution due to exploration and exploitation activities are a threat to the environment, particularly the mangrove ecosystem and agricultural land. World reserves of arable agricultural land are constantly diminishing and 25% of cultivated lands are affected by soil degradation due to man's activities. For example, a good percentage of oil spills that occurred on dry land between 1978 and 1979 in Nigeria affected farms in which crops such as rice, maize, yams, cassava, and plantain were cultivated. The primary concern of petroleum contamination of soil has been not only its effects on groundwater but on the germination and growth of some plants has also been reported. The recovery of soil fertility after an oil spill depends on several factors including the quantity spilled, the chemical composition of the crude oil, and the biodegrading potential of the microbial population in the area affected. Restoration of the fertility of agricultural land previously contaminated by oil is of great importance. For example, Adeniyi et al (1985) observed that the non-agricultural occupation of about 7% of the household heads in the Mkpannak area of Cross River state, Nigeria was due to the poor physical condition of the soil after an oil spill. Petroleum hydrocarbons (PHCs) "sterilize" the soil and prevent crop growth and yield for varying periods. The negative impact of oil exploration activities remains the major cause of the depletion of the Niger Delta's

vegetative cover and the mangrove ecosystem.

Different positions exist on methods to speed up the process just as there are different researchers. Atlas & Bartha, 1972 concluded that the disappearance of crude oil from seawater could be accelerated by the addition of deficient nutrients such as nitrogen and phosphorus or both others have suggested microbial seeding of oil spills since bacteria and fungi are the only biological ties that have the metabolic capability of utilizing petroleum carbon for cell synthesis. On the other hand, Christofis et al, 1998 posited that several agro-technical methods including tilling and loosening, watering, and addition of organic materials (straw, compost etc) and mineral fertilizer could decrease the contamination level by 30-40% due to the oxidation of easily degradable petroleum components.

As these findings become common knowledge, biodegradation of petroleum hydrocarbons has become an increasingly important method of treatment of the contaminant in polluted soils due to its advantages which include inexpensive equipment, environmentally friendly nature of the process, and simplicity (Nadeau et al., 1993). Hence the present study becomes necessary to broaden the horizon of existing knowledge on bioremediation and to investigate the factors that could be optimized for accelerated biodegradation, and this provides a veritable and cost-effective approach to the cleanup of contaminated soils in a low-income country like Nigeria where crude oil pollution of existing and potential agricultural lands is fast becoming a growing environmental problem.

Bioremediation technologies simply attempt to optimize micro-organisms' natural capacity to degrade/recycle by supplying essential inorganic limiting reactants and minimizing abiotic stress. Recommendations have been advocated for the microbial seeding of oil spills because bacteria and fungi are the only biological species that have the metabolic capacity of utilizing petroleum carbon for cell synthesis (Jobson et al, 1974).

Bioremediation techniques are versatile and can be utilized at various stages of treatment. Applications include the removal of contaminants from raw materials before processing, treatment of wastes before discharge treatment of effluent streams, and decontamination of soils, sediments surface water and groundwater. Many factors influence the bioremediation process and should be monitored. These include temperature, type of soil, pollutants type and concentration, nutrients, oxygen availabilities, and microorganism concentration on the impacted site. Therefore, there is a need to adjust some environmental conditions such as improving soil aeration, and monitoring and correcting the moisture and PH to stimulate the indigenous microorganism activity and to obtain the best pollutant removal (Sandro et al, 2005).

Crude oil pollution is harmful and dangerous to plants, animals, and humans hence the need to decontaminate sites polluted by crude oil. Physical and chemical methods of clean-up have proven to be ineffective in decontaminating polluted sites hence the need for a more effective and environmentally friendly method of clean-up through bioremediation. Bioremediation through natural attenuation is a slow process hence the need to stimulate the growth and activity of the microorganisms through the provision of nutrients in other to increase the rate of decontamination. It is important to provide environmentally friendly and cheaper means of decontaminating crude oil-polluted soils which would be vital to the restoration of polluted soils for agricultural purposes. In this study, the effect of decontamination of crude oil-polluted soil using NPK 15:15:15 and Urea was investigated: to determine the extent of degradation of the Total Hydrocarbon Content and to evaluate the effect of NPK 15:15:15 and Urea in enhancing the bioremediation process.

2. Materials and Methods

Samples

- **Soil**
  The soil type used for this project was loamy soil and was obtained from the back of the Chemical and Petroleum Engineering Department, University of Benin, Nigeria.

- **Crude Oil**
  The crude oil used for this study was obtained from Warri Refinery and Petrochemicals (WRPC), Warri, Delta State, Nigeria with a specific gravity of 0.92.
- **NPK 15:15:15**
  NPK 15:15:15 was obtained from a local shop at Ring Road, in Benin City, Edo state, Nigeria

- **Urea**
  Urea was obtained from a local shop at Ring Road, in Benin City, Edo state, Nigeria.

Sample Preparation

4kg of soil was weighed into four (4) different cells and was polluted with approximately 1L of crude oil and was well mixed.

Cell Sample

For this study, the following cells were used:

Cell 1 (A1): contained about 1L of Oil and 4kg Soil with the mixture continually stirred for uniformity. This served as the Control of this research.

Cell 2 (A2): contained the contents of cell 1 and 1kg of NPK 15:15:15 mixed thoroughly

Cell 3 (A3): contained the contents of cell 1 and 1kg of Urea mixed thoroughly

Cell 4 (A4): contained the mixture of 1L of Crude oil, 4kg soil, 500g NPK15:15:15, and 500g Urea thoroughly stirred for uniformity.

Determination of Parameters

Determination of pH

Weigh 20g of soil into a 100 ml beaker. Add 20 ml of distilled water. Thoroughly stir the mixture and allow it to stand for 30mins. Take the pH of the mixture using a pH meter

Determination of Residual Hydrocarbon Content (RHC)

Weigh 5g into a 100ml bottle. Add 25ml of n-hexane. Shake for 10mins and let stand covered. Filter read filtrate at 460 nm. The standard calibration curve was prepared by plotting the absorbance of standard (crude oil dissolved in hexane) of 1000, 2000, 4000, 6000, 8000, and 10000 ppm. A plot of concentration (mg/l extract) versus absorbance was made and the absorbance of an unknown sample

extract was converted to concentration by the equation below using the conversion factor obtained from the curve determined

RHC (mg/kg soil) = Absorbance x CF x DF x EV

Weight of soil

CF = conversion factor from absorbance to mg/l extract

DF = Dilution factor

EV = Extract volume of solvent (L)

Determination of Total Microbial Count (TMC)

Prepare a dilution of the soil sample by washing the soil with distilled water and diluting using the diluent already prepared (obtain 10^{-1}, 10^{-3}, and 10^{-6} dilutions). Assemble the Colony counting chamber by applying the cover glass. Add a few drops of Methylene blue solution to the water sample and dilute. With a standard loop place a loop full of water samples (including the various dilution) on the ruled area of the counting chamber. Allow the chamber to rest for 5mins. Examine under a microscope using a four mm lens (x 16 objective lenses) to count the bacteria in 50-100 squares selected at random, so that the total number of bacteria is about 500.

For each sample obtain triplicate counts divide the number of counts by the number of squares and multiply the result by the dilution factor and a constant k. This gives the number of organisms in milliliters of the given water sample.

3. Result and Discussion

Table 1: Variation of pH with time.

	Urea	NPK	Urea + NPK	control
pH				
Week 1	5.73	6.04	5.89	5.28
Week 2	5.84	6.01	5.77	5.34
Week 3	5.86	6.03	5.94	5.52
Week 4	5.71	6.08	5.91	5.57
Week 5	6.09	6.04	5.92	5.66
Week 6	6.03	6.07	6.04	5.68
Week 7	6.12	6.02	6.07	5.70
Week 8	6.06	6.05	6.02	5.71

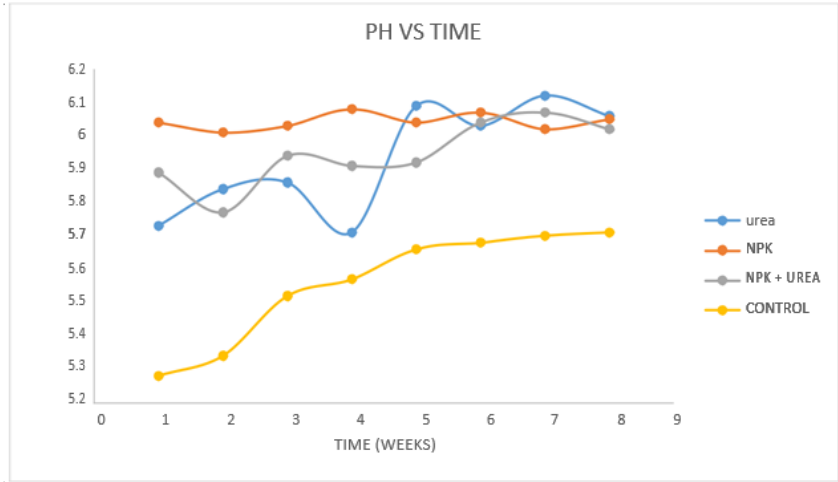

Fig. 1: Variation of pH with time.

Table 2: Variation of Residual Hydrocarbon Content (RHC) with time.

	Urea	NPK	Urea + NPK	control
RHC (mg//kg)				
Week 1	6282.62	6328.64	6317.18	6422.53
Week 2	5516.33	5489.54	5473.97	6101.27
Week 3	4789.28	4682.53	4582.67	5689.68
Week 4	4023.72	3971.88	3841.59	5183.44
Week 5	3218.60	3092.37	2903.43	4672.62
Week 6	2132.27	1984.54	1889.65	4077.81
Week 7	1565.38	1496.47	1458.36	3901.12
Week 8	897.96	809.55	790.83	3577.81

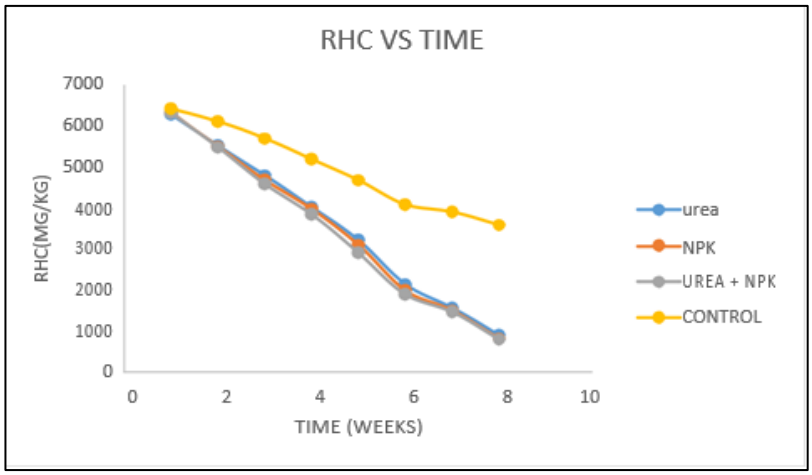

Fig. 2: Variation of Residual Hydrocarbon Content (RHC) with time.

Figure 2 shows the amount of residual hydrocarbon content over a period of eight weeks. Bioremediation of petroleum and hydrocarbons in the environment is a complex process where quantitative and qualitative aspects depend on the nature and amount of hydrocarbon present. It can be seen from Figure 2 that the total hydrocarbon content decreases with time for the various applied nutrient (bio-stimulant). After eight weeks of remediation, the percentage of total hydrocarbon degradation for the treatment cell were 85.71%, 87.21%, and 87.48% for the cells containing 1kg urea, 1kg NPK, and 500kg mixture each of urea and NPK respectively, while the control has a degradation rate of 44.29% of total hydrocarbon content. This signifies that there was an improvement in the degree of remediation offered by the various masses of solid waste (compost) used since the rate of degradation of hydrocarbon content in the control is very small. It is also evident that the amount of amendment applied affected the rate of bioremediation positively.

Variation of % Degradation with Time

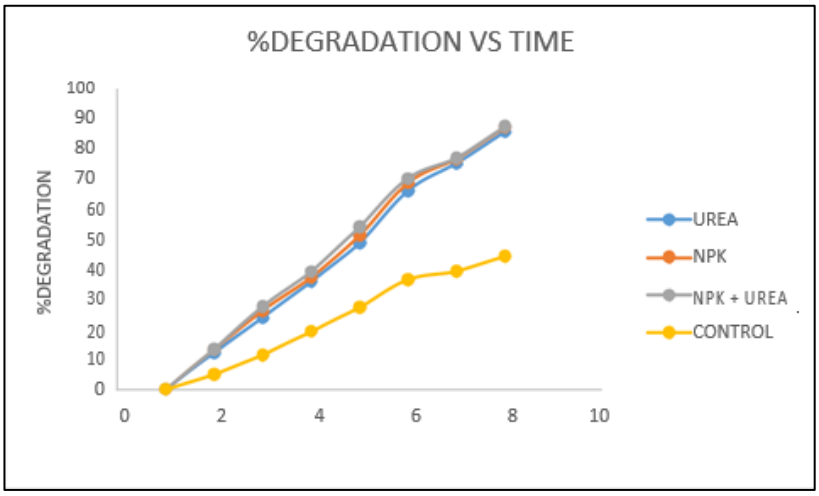

Fig. 3: Variation of % Degradation with Time.

There was a marked decrease in total hydrocarbon content in all the treatments except the control which recorded a value of 44.29%. The combination of Urea and NPK gave the highest % degradation of 87.48%. This was closely followed by amendment using NPK alone with a % degradation of 87.21%. That urea alone has a % degradation of 85.71%. This shows that the addition of amendments has helped in degrading the hydrocarbon content in the soil considering the % degradation in comparison with the control.

Variation of Total Microbial Count (Cfu/g) with Time

Table 4: Variation of Total Microbial Count (TMC) with time.

	Urea	NPK	Urea + NPK	Control
TMC/ml				
Week 1	$6X10^6$	$6X10^6$	$5X10^6$	5×10^4
Week 2	$5X10^6$	$6X10^6$	$7X10^6$	4×10^4
Week 3	$6X10^6$	$7X10^6$	$6X10^6$	6×10^4
Week 4	$7X10^6$	$7X10^6$	$6X10^6$	6×10^4
Week 5	7×10^6	8×10^6	8×10^6	7×10^4
Week 6	$8X10^6$	$9X10^6$	$9X10^6$	8×10^4

Week 7	$8X10^6$	$8X10^6$	$8X10^6$	8×10^4
Week 8	$9X10^6$	$9X10^6$	$9X10^6$	9×10^4

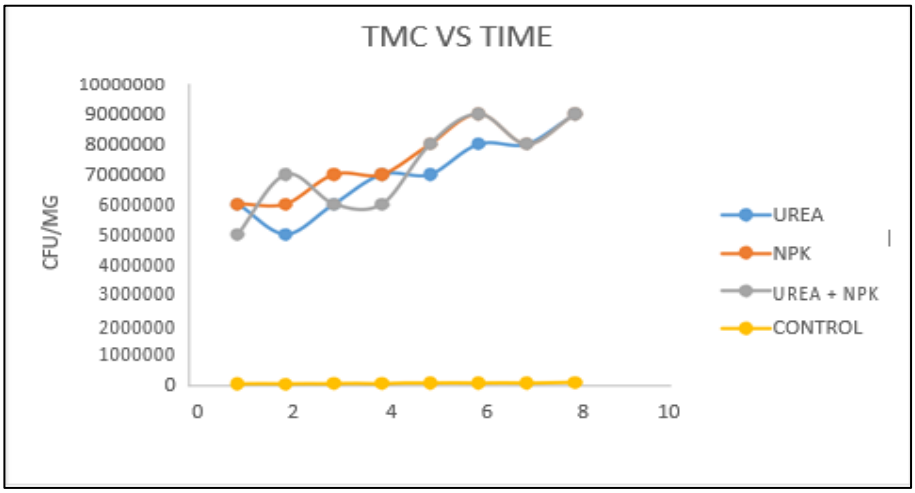

Fig. 4: Variation of TMC with time.

The experimental results as shown in the table and graph plotted indicate a constant increment in the number of microorganisms present in the different cells. Considering the control, initially, the amount of naturally occurring microorganisms was 5 x 10^4, then there was a drop to 4 x 10^4 this is because the microorganisms were trying significantly to get acclimatized with the new system, and the stronger microbes adapt to the system trumping on the weaker microbes, while the weaker microbes die and give way hence reducing the number of microorganisms. In the weaker cells there are variations in weeks 1-3 as in alternating values but from week 4 we a steady rise in the total microbial count. This increment can be attributed to the fact that once the microbes are adapted to the system and there is the availability of substrate (food source) in this case crude oil, the microbes break down the crude oil into less toxic substances (CO_2 and H_2O) that is environmentally acceptable.

Conclusively, it is observed that the control has the least Total Microbial Count (TMC) showing that the rate of bioremediation was enhanced by the amendments.

4. Conclusion

From the study, it is observed that bioremediation offers a cheaper, more effective, and environmentally friendly way of treating crude oil-polluted soils, which is a crucial problem facing Nigeria and countries where crude oil is exploited. The effect of the various nutrients (biostimulants) and a combination of them from the results show that there was an explicit improvement in the contaminated soil over the eight weeks when compared with that undergoing natural attenuation.

The cell containing a combination of Urea and NPK 15:15:15 gave the highest % degradation (87.48) after eight weeks in comparison with other cells indicating its usefulness in bioremediation and this value complied with that of FEPA closely. Comparing the control and the other cell in the above-discussed result shows that biodegradation can also take place naturally but will take a longer time to meet the satisfied conditions by FEPA. It is highly recommended that the study into bioremediation of crude oil-contaminated soil which seems to pose a major threat to the ecosystem be encouraged in tertiary institutions to enhance the discovery of more effective methods and various combinations for the treatment of polluted soils. Equipment to enhance constant research by students, lecturers and researchers should be made readily available in the laboratories. The technique used in this study was Biostimulation and Natural attenuation, other techniques such as Bioaugmentation should be experimented on to find better alternatives.

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