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Assessment of the Biodegradable Ability of Palm Bunch Enhance Stimulant for the Bioremediation of Crude Oil Contaminated Soil

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Abstract

The work is focused on the application of palm bunch enhance stimulant (PES) and modified crude oil dispersant (MCD) in the remediation of crude oil polluted soil. The precursor MCD was synthesized and used in the synthesis of PES. Batch remediation studies were carried out on the polluted soil. Concentration of PES/MCD (mg/l), concentration of crude oil (mg/l) and depth of soil (cm) were the factors considered. The results showed that the % total petroleum hydrocarbon (TPH) removal increased as the concentration of PES increased from 400 mg/l to 500 mg/l. Increased concentration of crude oil from 100 mg/l to 300 mg/l also resulted to decreased % TPH removal. The % TPH removal decreased as the soil depth increased from surface to 4cm depth. The PES was found to be more efficient in the removal of TPH than MCD due to the presence of nutrient to support microbial growth. Application of PES to stimulate the degradation of crude oil polluted soil can enhance the degradation of crude hydrocarbon through increased bioavailability of petroleum hydrocarbon to soil microorganism.

Keywords: Bioremediation, biostimulant, PES, palm bunch, petroleum

1.0 Introduction

Oil spill incidents have occurred in different parts of the World in both aquatic and terrestrial environments. These are caused by drilling, refining, transportation of crude oil products (Oludele et al., 2021). Most of these spills are associated with negligence and sabotage, corrosion of pipes, and oil tankers accidents (Wang et al., 2011). Since crude oil derived products continue to be a major source of energy for most homes and industries, their entry into the environments either by accident or negligence affects humans, plants and animal (Wolicka et al., 2009). Several methods such as solvent extraction, incineration, bioventing, biopile, composting, the use of dispersants, absorption and burial in secure landfills have been employed in the remediation of contaminated sites. These methods have been reported to be inefficient, expensive, non-biodegradable, toxic to plants and animals (Johnsen et al., 2005, Zahed et al., 2011). There is therefore the need to treat crude oil spill using an eco-friendly, green, facile, non-toxic, low-cost and biodegradable methods. Bioremediation is a contamination control technology that employs the mechanism of biodegradation to clean up polluted environment by utilizing metabolic activities of micro-organisms to convert various toxic substances into harmless product (Ita&Osalodion, 2021). It offers reduced environmental risks as it is a natural process that depend on microorganisms to degrade the contaminants in soil and water sources. It is the use of microorganism to breakdown contaminants such as crude oil into carbon dioxide, water and other less harmful substances. Furthermore, bioremediation technology is believed to be non-invasive and relatively cheaper than other methods (April et al., 2000).

However, the bioremediation process has got some limitations. It is a slow process (Sobika et al., 2021), and therefore requires weeks to months to complete the process. Hence, when immediate cleanup is required, bioremediation may not be feasible. To speed up the bioremediation process addition of nutrients (stimulant) are very essential (Wang, 2011). Therefore the use of biostimulant is necessary to add additional nutrients to the microbes in order to stimulate the activity of microorganisms that break down complex organic compounds to harmless compounds. The application of PES to remediation of contaminated site is therefore a good alternative.

Researchers have reported the use of carob kibbles, sugarcane bagasse, sugarcane molasses, wheat straw, banana skin, yam peel, saw dust, spent brewing grain, rice husk, and coconut shell as bio stimulating agents (Hamoudi-Belarbi et al 2017; Alotaibi et al., 2018; Abioye et al., 2010; Molina-Barahona et al., 2004) . Other reported works focused on the use of other toxic and less effective solvents, and inorganic mineral elements for enhancement of bioremediation. However, very little studies have focused on the mineral element from plants as potential bio-stimulating agent. The indiscriminate discharge of palm bunch into the environment is a public health concern and thus its use for crude oil bioremediation purposes will help arrest this ecological disaster and further lower the cost of oil spill cleanup since it is readily available nationwide and at no cost. The use of enhanced solvent (paraffin oil) together with nutrients from plant materials help to stimulate the bioremediation process and provide better bioavailability of the contaminants to the microbes. The hybrid PES is non-toxic, eco-friendly, biodegradable and cheap, and is expected to reduce the remediation time greatly.Review of literatures show that no work has been reported on the effect of dispersant on the depth of contaminated soil.

Treatment of contaminated soil by the use of dispersants and bio-stimulants at the surface of soil have been reported by many authors. However, there is currently no report on the performance of such treatment on the layer of soil below the topmost soil. When there is a soil pollution, crude oil usually sips into the soil. During bioremediation, the dispersant and or biostimulant are applied on the surface of soil. The performance of the dispersant or biostimulant are usually measured on its ability to degrade the crude oil on the topmost soil. Currently, there is no reported work on the performance of dispersant or biostimulant on the layer of soil below the topmost soil.Therefore, this work seeks to proffer a solution to these problems identified. The work involves synthesis of PES, characterization of the PES, determination of the biostimulating efficiency of PES and determination of the influence of process variables on the use of PES for remediation.

2.0 Materials and method

2.1 Material collection

Pure and analytical grade chemicals were used in all the experiments including. The chemicals were purchased from Bridge Head Market Onitsha, Anambra State. Crude oil was collected from Nigeria National Petroleum Corporation, (NNPC), Port Harcourt, Rivers State.

2.2 Preparation of Materials for the PES synthesis

2.2.1 Preparation of modified crude oil dispersant (MCD)

62.5g of sodium hydroxide (caustic soda) was dissolved in one liter of water and allowed to stand for 24 hrs. 62.5g of sodium bicarbonate (soda ash) was also dissolved in 1 liter of water and kept for 24 h. 62.5g of nitrosol and 62.5g of sodium laurel sulphonate (SLS) were also dissolved in 1 liter each of water and kept for 24 h. 250 mL of sulphonic acid and 123 mL texapon were mixed properly and dissolved with 2 liter of water and kept for 20 minutes. The sulphonic acid and texapon mixture were added into the reactor containing nitrosol and stirred properly. Thereafter, the fermented caustic soda, soda ash and SLS were added in that order with continuous stirring. 260 mL of foam booster was added into the mixture, followed by addition of 4 liters of water. All the mixtures were stirred properly and allowed to stand for 24 h before use.

2.2.2 Preparation of Palm Bunch Ashes

Alkali was extracted from palm bunch according to the modified procedure by Ogunsuyi and Akinnawo, (2012). Palm bunch wastes were collected from palm oil processing facility at AmaenyiAwka, Anambra State, and sun dried. It was thereafter oven-dried at a temperature of 105^oC for two days to ensure sufficient removal of moisture from the sample. The bone-dried bunchwas charred for 3h to ensure uniform combustion. The resultant charred bunch was further ashed in a furnace at a temperature of 550^oC for 8h. The ashed sample was crushed properly in a mortar and then sieved with analytical sieve of mesh size 75μm to obtain uniform particles size. 100g of the ash was weighed out into a 2000 ml round bottom flask containing 500 mL of distilled water. The flask was placed on a heating mantle and boiled to about 100^oC for 4 h. Thereafter, the flask was allowed to stand for 48h and the content was filtered using poplin cloth and re-filtered with Whatman filter paper to obtain clearer extract. The filtrate was poured into an air tight container for further use.

2.2.3 Synthesis and modification of Hybrid bio-stimulant (PES)

400 mL of MCD produced was mixed properly with 100 mL of PBA filtrate in a reactor and allowed to stand for 10 minutes. 0.5g of NPK fertilizer, 0.5 g of Na_2SO_4, 50 mL of paraffin oil were added into the mixture and stirred properly for 20 minutes. The resultant PES was left to settle for 24 h at room temperature.

2.3 Determination of total petroleum hydrocarbons (TPH)

The methods applied is the modified ASTM Method D 7066–04.UV/VIS Spectrophotometer was used to determine the absorbance of the samples. TPH extraction and analysis was carried out following the laboratory manual adopted by Macgille (2000). A standard calibration curve was first constructed. A standard concentration of 1000ppm of crude oil in hexane was prepared as standard stock. Working standards of 10, 20, 40, 60, 80 and 100 ppm was prepared from the standard stock. The absorbance of these concentrations were obtained with T-60 UV/Visible spectrophotometer at a wavelength of 420. The values of absorbance obtained were plotted against concentration to obtain the calibration curved used in this study. For every analysis, 50ml of the sample solution was taken in a 150ml separating funnel to which 10ml of hexane was added, shaken manually for 2 minutes and allowed to stand for 20 minutes without the stopper. The water layer was drained off, hexane layer collected in quartz curvet and read using T-60 UV/Visible spectrophotometer at a wavelength of 420 nm. The absorbance obtained was converted to concentration by comparing it with the calibration curve of hexane as obtained above. The TPH at any point, was determined using eqn. (1.0) (Latinwo& Agarry,2015).

$$\%TPH = \frac{TPH_o - TPH_t}{TPH_o} * 100 \qquad (1.0)$$

Where:
TPHo = Total Petroleum Hydrocarbon at day 0 (mg/kg)
TPHt = Total Petroleum Hydrocarbon at any day t (mg/kg)

2.4 Biostimulation efficiency

The efficiency of the PES/MCD was evaluated using Eq. 2.0 (Agarry et al., 2013, Umeojiako et al., 2019)

$$\%\,B.E = \frac{\%TPH_{(s)} - \%TPH_{(U)}}{\%TPH_{(s)}} * 100 \qquad (2.0)$$

Where $\%\,TPH_{(s)}$is the removal of crude oil in the amended soil, and $\%\,\mathrm{TPH}_{(u)}$the removal of crude oil in the unamendedsoil.

2.5 Batch bioremediation studies

2.6 Effect of PES and MCD concentration on contaminated soil

This was carried out according to the modified procedure by Chukwuemeka (2016). Seven plastic containers were used as bioreactors. 200 mg/L concentration of crude oil was prepared. 100 mL of 200 mg/L of the crude oil was used to artificially pollute 500 g of soil. The soil samples and the crude oil were properly mixed to obtain a uniform mixture. 50 mL of 400, 450, and 500 mg/l concentration of the PES was prepared and added into three of the bioreactors, respectively. 50 mL of the same concentration of MCD was added into another three different bioreactors, respectively. The remaining one reactor served as the control and contained only polluted soil. 50 mL of water was added into each bioreactor. The initial concentration of crude oil was kept constant at 200 mg/l and the process was carried out at room temperature. The TPH was measured every two days starting from day zero when the experiment started to about day 30 when the bioremediation experiment was halted. 50 mL of water was added into each reactor every three days to replenish the evaporated water and to keep the process going.

2.7 Effect of Crude oil Concentration

This was carried out according to the modified procedure by Chukwuemeka (2016). Seven plastic containers were used as bioreactors. 100, 200, and 300 mg/l of crude oil was prepared. 100 mL each of the different crude oil concentration prepared was added into three bioreactors containing 500 g each of the soil, respectively. 50 ml each of 500 mg/l concentrations of PES was added to the first three bio-reactors while the same concentrations of MCD was added into the second three bio-reactors, respectively. The last bioreactor served as the control and contains only contaminated soil. 50 mL of water was added into each bioreactor to keep it moisten. Temperature and concentration of PES/MCD were kept constant. The TPH was measured every two days starting from day zero when the experiment started to about day 30 when the bioremediation experiment was halted. 50 mL of water was added into each reactor every three days to replenish the evaporated water and to keep the process going.

2.8 Effect of depth of soil.

This experiment was done according to the modified procedure by Santhaveerana et al, (2016).The soil used in this research was excavated at 5 cm depth from the ground at a land in NnamdiAzikiwe University, Awka. The soil was air dried, pulverized using mortar and sieved using a mechanical shaker. The soil retained on 75 μm sieve was used for this work. Three sets of graduated and transparent bioreactors of 10 cm height was used for the experiments. Each bioreactor was filled with soil to 7 cm depth. 100 mL of 200mg/l of crude oil was used to artificially pollute the soil in each of the three bioreactors. 50 mL of water was measured into each bioreactors. 50 mL of 500 mg/L of PES and 500 mg/L of MCD was added into the first and second bioreactors. The third reactor serve as control (no PES or MCD was added into it). The set up was allowed to remediate for 30 days at room temperature. The TPH was measured at 0, 2, 3, and 4 cm depth every two days starting from day zero when the experiment started to about day 30 when the bioremediation experiment was halted. 100 ml of water was added to the bioreactors every five days to replenish the moisture that was lost through evaporation.

3.0 Results and discussion

3.1 Characterization results

The results of AAS on PBA is shown in Table 1.0. According to Table 1.0, PBA contains 0.735, 13.022, 11.222, 92.890, 12.71 and 12.86 mg/l of magnesium, potassium, calcium, sulphate, phosphate and nitrate, respectively. According to Das & Chandran, (2011), a bacterial cell is 50 percent carbon, 14 percent nitrogen, 3 percent phosphorus, 2 percent potassium, 1 percent sulfur and 0.5 percent each of calcium and magnesium. From the results obtained in Table 1.0, it could be observed that PBA contains appreciable quantity of each of potassium, sulphate, calcium required for cell growth and development. In bioremediation, the compound that receives electrons (is reduced) in the energy-producing oxidation-reduction reactions that are essential for the growth of microorganisms and bioremediation are called electron acceptors. Common electron acceptors in bioremediation are oxygen, nitrate, sulfate, and iron. It could be seen from Table 1.0 that PBA contains an appreciable amount of sulphate (an electron acceptor), which was why it was chosen in this work. Table 2.0 shows the physical properties of the dispersant (MCD) and the biostimulant (PES). According to Table 2.0, the density of MCD is 1.0456 kg/m^3 while the density of PES is 1.078 kg.m^3. The viscosity of MCD and PES are 1944 and 1032 MPa.s, respectively. It shows that the MCD was more viscous than the PES. This is because during the preparation of PES, the more viscous MCD are mixed with paraffin, palm bunch extract and some quantity of water. All these contributed to the lower viscosity of PES compared to MCD.

Table 1.0: Characterization of PBA

S/N	Parameters	Composition (mg/l)
1	Magnesium	0.735
2	Potassium	13.022
3	Calcium	11.222
4	Sulphate	92.890
5	Phosphate	12.710
6	Nitrate	12.860

Table 2.0: Physical Properties of PES and MCD

S/N	Parameters	MCD	PES
1	Density(kg/m^3)	1.0456	1.078
2	Viscosity(MPa.s)	1944	1032
3	Refractive Index	1.344	1.343

3.2 Influence of concentration of PES/MCD

The influence of concentration of PES/MCD on the remediation of crude oil polluted soil and water was studied at these conditions: concentration of crude oil (200 mg/l), depth of soil (1 cm), and room temperature (30 ^oC). The results are presented in Figs. 1-4. Fig. 1.0 shows the plot of concentration of PES (mg/l) against the time of remediation (days). It shows that as the concentration of PES increased from 400 mg/l to 500 mg/l, the percentage removal of TPH increased. At zero day of remediation, the percentage TPH remaining in the soil were 98.228, 95.555, 92.886 and 99.912 % for 400 mg/l, 450 mg/l, 500 mg/l and UAS, respectively. The percentage of crude oil remaining on the soil kept decreasing as the time increased from zero to 18 days. On the 8th day of remediation, the percentage of crude remaining in the soil were 39.332, 37.938, 31.134 and 95.11% for 400 mg/l, 450 mg/l, 500 mg/l and UAS, respectively. However, on the 18th day of remediation, the remaining crude oil in the soil was 16.122, 12.012, 7.578 and 86.149 % for 400 mg/l, 450 mg/l, 500 mg/l and UAS, respectively.Fig. 3.0 also show the influence of MCD concentration on the remediation of crude oil polluted soil. It also revealed that increasing the concentration of MCD results to increase in the percentage of crude oil removed from the soil. However when the percentage removal of crude oil from soil by PES was compared to that of MCD, it was observed that PES performed better. At day two of remediation, the percentage of crude oil remaining after remediation by 400, 450 and 500 mg/l of PES were 88.564, 86.461 and 80.362 %, respectively. At the same time and under the same operating conditions, the percentage of crude oil remaining after remediation by 400, 450 and 500 mg/l of MCD were 92.104, 87.138, 82.175 % respectively. At day 18 of the remediation, the percentage of crude oil remaining in soil after remediation by 400, 450 and 500 mg/l of PES were 16.122, 12.012 and 7.578 %, respectively. While the percentage remaining after remediation by 400, 450 and 500 mg/l of MCD were 28.101, 26.025 and 19.134 % respectively. Increase in the concentration of PES increases the amount of nutrient available for the remediation of the polluted soil leading to faster degradation of the substrate (crude oil). Organic contaminants usually serve two purposes for the organisms: they provide a source of carbon, one of the basic building blocks of new cell constituents.

Organic contaminantsalso provide electrons, which the micro-organisms can extract to obtain energy. These microbes obtain energy by catalyzing energy-producing chemical reactions that involve breaking chemical bonds and transferring electrons away from the contaminant and utilizing the electrons in the formation of new cells. Since the PES contains enough external electron donor (Na_2SO_4), it could reduce the concentration of crude oil in the soil with increase in its concentration. It could also be seen from Fig. 1.0 that UAS had the least removal efficiency because its use in the remediation took place in the presence of no external nutrient. The presence of such nutrient as nitrogen, phosphorus, potassium, sulfur, iron, calcium, magnesium, and chloride supports the growth of micro-organisms during bioremediation. If any of the elements essential for cell building is in limited supply, it result incompetition for nutrients within the microorganism communities. These competitions between the microorganisms may limit overall microbial growth and slow contaminant removal. This explains the reason why bioremediation system must be designed to provide the proper concentrations and ratios of these nutrients if the natural habitat does not provide them. Bioremediation is a fairly slow process and therefore requires weeks to months to complete the process. Hence, to speed up the bioremediation process addition of nutrients (stimulant) are very essential (Wang, 2011).

Therefore the use of PES is necessary to add additional nutrients to the microbes in order to stimulate the activity of microorganisms that breaks down complex organic compounds to harmless compounds. According to Fig. 2.0, the percentage degradation of crude oil at the end of 18 days of remediation were 80.583, 82.99, 85.99 and 12.81 % for 400 mg/l, 450 mg/l, 500 mg/l and UAS, respectively.Fig.4.0 shows the percentage degradation of crude oil in the soil by MCD. The percentage degradation of crude oil by MCD (Fig. 4.0) on day 18th of the degradation were 68.97, 69.47, 74.90 and 13.85 for 400 mg/l, 450 mg/l, 500 mg/l and UAS, respectively. It shows that addition of nutrient (PES) performed better than both MCD and the unamended soil (UAS) in the degradation of the crude oil. The addition of NPK, PBA and Na_2SO_4 to PES increased the amount of nutrients which in turn brought about increase degradation of the crude oil. Oludele et al., (2021) and Ijah&Safiyanu, (1997) have reported similar findings.

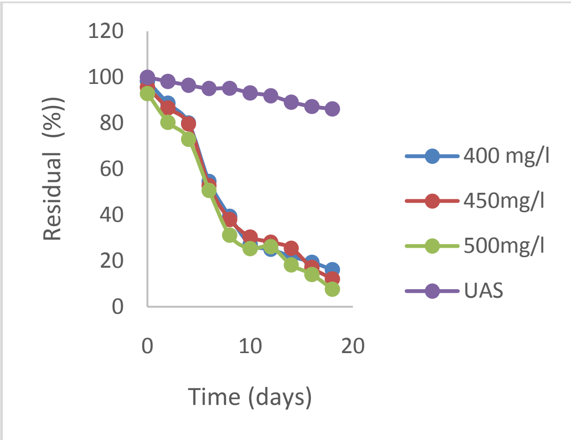

Fig. 1.0: Influence of PES concentration on soil remediation

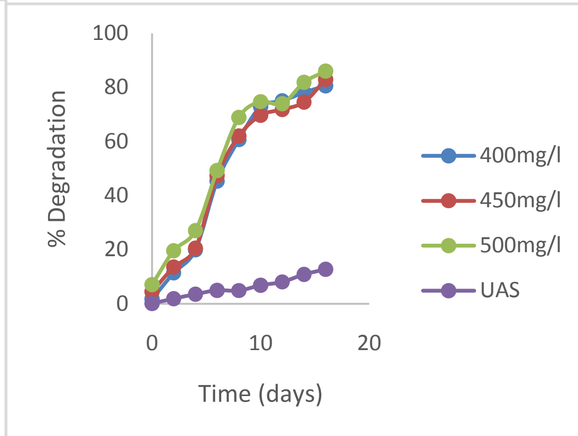

Fig. 2.0: % Degradation of crude oil using PES for soil remediation

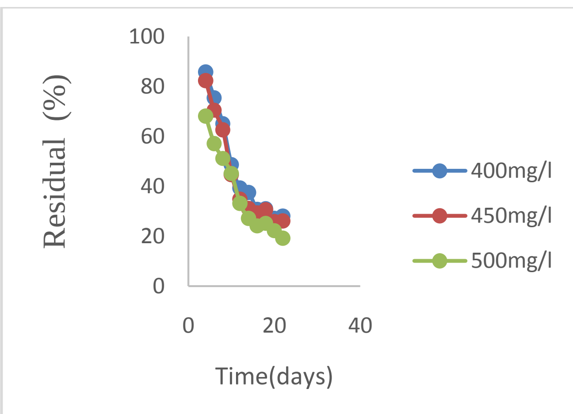

Fig. 3.0: Influence of MCD concentration on soil remediation

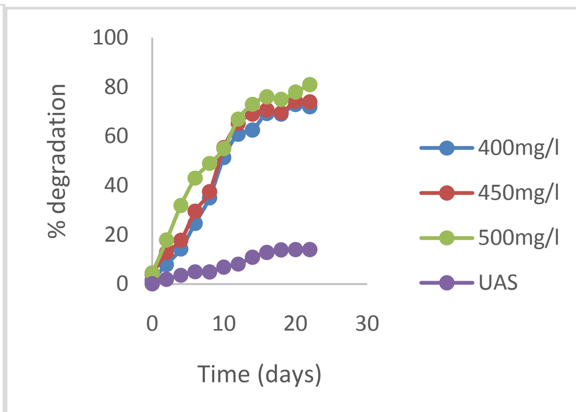

Fig. 4.0: % Degradation of crude oil using MCD for soil remediation

3.3 Influence of concentration of crude oil

The influence of initial concentration of crude oil on the remediation of crude oil polluted soil and water was studied at these conditions: concentration of PES/MCD (450 mg/l), depth of soil (1 cm), and room temperature (30 ^{o}C). The results are shown in Figs. 5.0-8.0. Fig. 5.0 shows the plot of initial concentration of crude oil (mg/l) against degradation time (days) using PES. The concentration of crude oil remaining after two days of remediation were observed to be 85.722, 88.564 and 99.023 % for 100, 200 and 300 mg/l initial concentration of crude oil in soil, respectively (Fig. 5.0). At day 18, the concentration of crude oil remaining in the soil were observed to be 12.47, 19.421 and 24.005 % for 100, 200 and 300 mg/l initial concentration of crude oil in soil, respectively.Fig 7.0 shows the plot of initial concentration of crude oil against remediation time for MCD induced soil remediation.At day two of remediation, the quantity of crude oil remaining in the soil were 90.971, 93.105 and 95.238 % for 100, 200 and 300 mg/l initial concentration of crude oil, respectively.

However, the percentage of crude oil remaining at day 18 were 24.194, 25.507 and 48.497 % for 100, 200 and 300 mg/l initial concentration of crude oil, respectively. This revealed that increase in concentration of crude oil in the soil results to decreased efficiency. It takes longer time for microbes to degrade higher concentration of crude oil. At lower concentration, the number of crude oil molecules distributed in the soil is small. When acted upon by microbes, the percentage removal of the crude oil from soil increases in lower initial crude oil concentration than higher initial concentration. This could be further confirmed in Fig. 6.0 where the percentage degradation of the crude oil by PES for the different initial concentration of oil were 87.53, 80.579 and 75.995 %, respectively at 18th day.

Fig.8.0 also shows the percentage degradation of crude oil by MCD. At day two, the percentage degradation of crude oil by MCD was 9.02, 6.89 and 4.60 % at 100, 200, and 300 mg/l, respectively. This increased to 75.80, 74.49 and 51.50 % at 100, 200, and 300 mg/l, respectively, on day 18th. It was observed that it took longer time to degrade the crude oil in soil using MCD than PES due to the presence of much more additional nutrient in PES than in MCD. This additional nutrient aid faster growth and multiplication of the microbes which in turn is responsible for the biological degradation of the pollutant (crude oil).

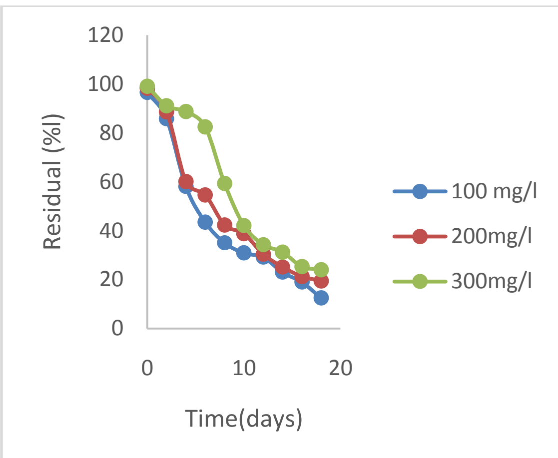

Fig. 5.0: Influence crude oil concentration on remediation of soil by PES

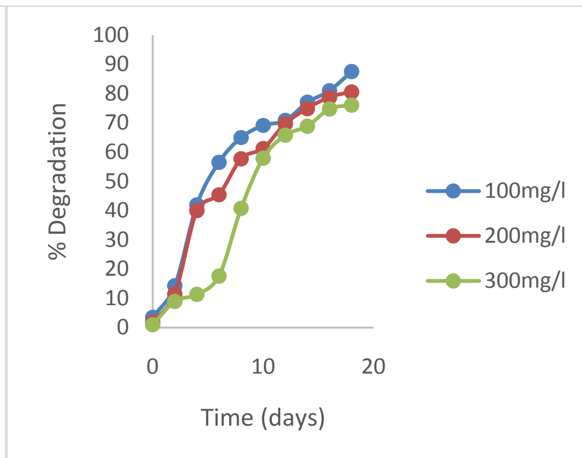

Fig. 6.0: % Degradation of crude oil in soil using PES

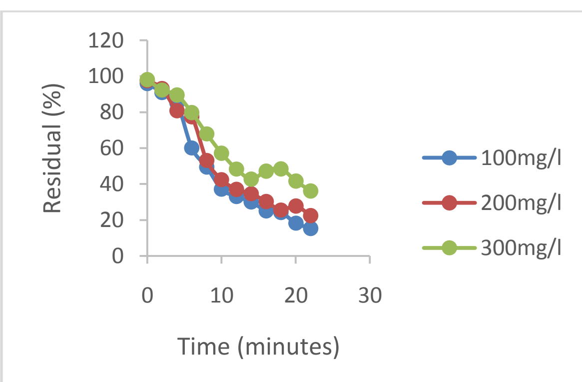

Fig. 7.0: Influence crude oil concentration on remediation of soil by MCD

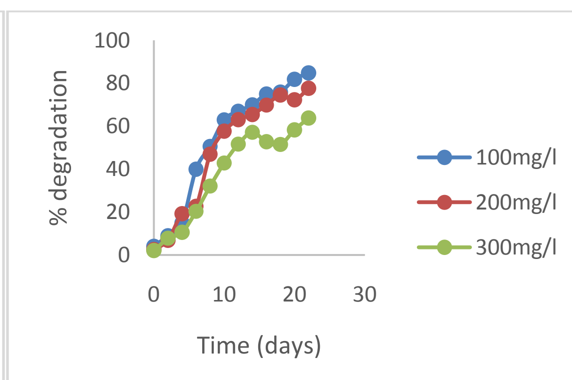

Fig. 8.0: % Degradation of crude oil in soil using MCD

3.4Influence of depth of soil

The influence of depth of soil on the treatment of crude oil polluted soil using PES/MCD was studied at these conditions: concentration of PES/MCD (450 mg/l), concentration of crude oil (200 mg/l) and room temperature (30

^{o}C).The results are presented in Figs. 9.0-12.0. In Fig. 9.0, the influence of depth of soil on the remediation of soil by PES shows that increase in the depth of soil reduces the efficiency of the treatment. It shows that the soil at the surface experienced a more removal efficiency than those at 1 cm depth and those at 2 cm depth received more removal efficiency than those at 3cm depth and so on. At day two of the remediation, the percentage of crude oil remaining in the soil were 94.299, 96.564 and 97.059 for 2.0, 3.0 and 4.0 cm depth, respectively. The percentage of crude oil remaining in the soil however decreased to 4.469, 11.167 and 21.71 % for 2.0, 3.0 and 4.0 cm, respectively on day 20th.Fig. 11.0 shows the influence of depth of soil on the remediation of soil by MCD. At day two of the remediation, the percentage of crude oil remaining in the soil were 95.807, 97.105 and 98.402 % for 2.0, 3.0 and 4.0 cm respectively. Day 20th show the percentage of oil remaining to be 28.736, 31.098 and 35.675 % for 2.0, 3.0 and 4.0 cm respectively.

According to Fig. 10.0, the percentage degradation of crude oil in the soil using PES at 20th day were 95.529, 88.833 and 78.29 % for 2.0, 3.0 and 4.0 cm, respectively. It shows that the PES employed could go beyond surface treatment to affect layer of soil 4 cm below surface level.

According to Fig. 12.0, the percentage degradation of the crude oil by MCD at day 20 were 71.253, 68.902 and 64.325 % for 2.0, 3.0 and 4.0 cm respectively. When Fig. 10.0 and 12.0 were compared, it shows that it took lesser time to degrade the crude oil in soil by application of PES. At 20th day, the percentage degradation of crude oil in soil were 95.529, 88.833 and 78.29 % for 2.0, 3.0 and 4.0 cm, respectively for PES. However, at 20th day, the percentage degradation of crude oil by MCD were 71.253, 68.902 and 64.325 % for 2.0, 3.0 and 4.0 cm, respectively. The presence of more nutrients in PES caused more degradation as observed.

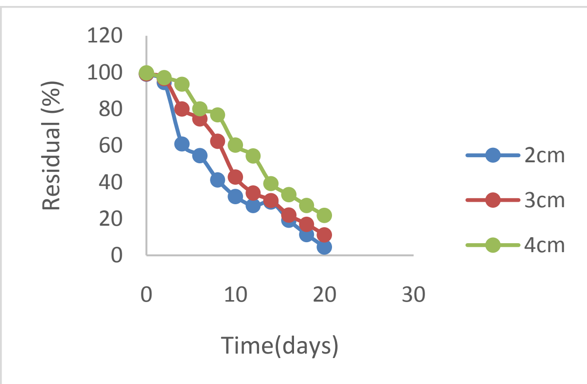

Fig. 9.0: Influence depth of soil on remediation of soil by PES

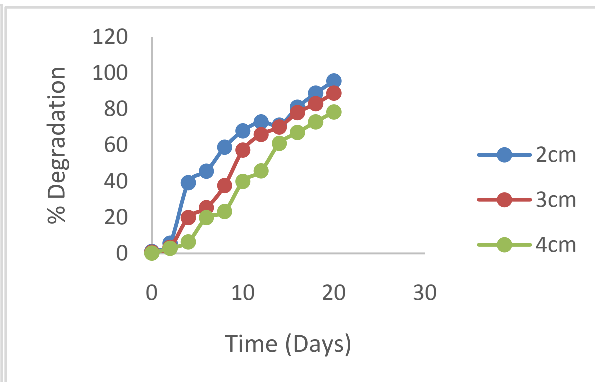

Fig. 10.0: % Degradation of crude oil in soil using PES at different depth

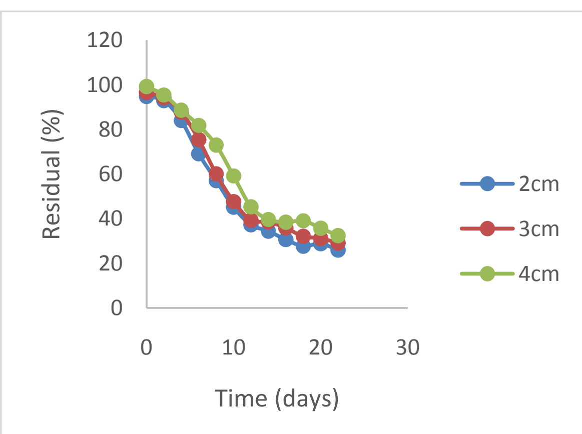

Fig. 11.0: Influence depth of soil on remediation of soil by MCD

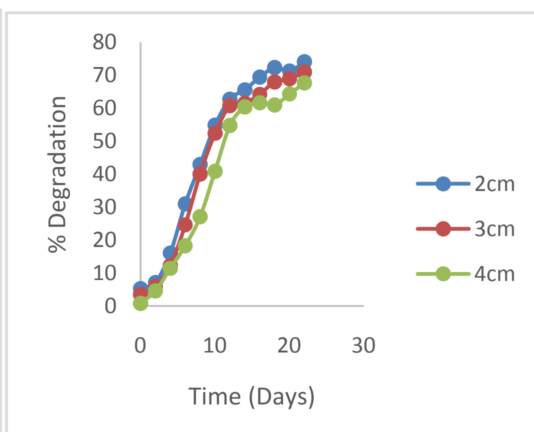

Fig. 12.0: % Degradation of crude oil in soil using MCD at different depth

3.5Bio stimulation Efficiency

Eq. 2.0 was used to calculate the bio-stimulation efficiency (BE) of the PES and MCD. The bio-stimulation data obtained was plotted against remediation time and the results are presented in Figs. 13.0 and 14.0. The data were fitted to polynomial equations and various models were developed to predict the bio-stimulation efficiency of PES and MCD in soil and the results are presented in Eqs. 3.0, and 4.0. According to Fig. 13.0, the bio-stimulation efficiency of PES in soil was higher at the early stage of the process indicating that PES is more effective during the early stage of bioremediation. However, Fig. 14.0 indicates that MCD had a lower stimulation efficiency at the early stage of the process due to limited nutrient to drive the degradation process. The optimum bio-stimulation efficiency of PES in soil (Fig. 13.0) occurred at day 8 with BE values of 87.89 %. However, the BE of MCD in soil (Fig. 14.0) showed a relatively lower values at the beginning of the process and peak toward day 16. The optimum BE value recorded at this period is 76.465 %. Coefficient of determination (R^2) was used to establish the best fit models. R^2-value indicates the magnitude of variance in the dependent variable that can be predicted from the independent variable. The R^2 value of 0 suggests non-prediction and R^2 of 1 indicates a perfect prediction. According to the models developed, PES in soil (Eq. 3.0) had R^2 values of 0.9318. It shows that the models developed gave 93.18 % prediction of the dependent variable by the independent variable. The high R^2 values obtained for this is good.

However, R^2 obtained for MCD in soil (Eq.4.0) was 0.6691. This showed that the model developed for MCD in soil predicted 66.91 % of the dependent variables by the independent variables. This means that the model developed for MCD cannot sufficiently explain the behavior of the remediation system. The low values of R^2 for MCD in soil is not good and show how poorly the MCD performed in the stimulation of the bio-remediation process. These equations will help to predict the bio-stimulation efficiency of PES and MCD at any given time. Applications of these model equations could help to design a bioremediation treatment system to clean up a crude oil polluted soil.

The difference in the bio-stimulation efficiency of PES and MCD may be attributed to their specific nutrient composition, content and bioavailability. The addition of bio-stimulating agents to soil has been reported to increase oxygen diffusion and mineral nutrient availability as well as carbon source quality and mechanical support surface for bacterial adsorption. It also improves soil physicochemical characteristics by speeding up microbial adaptation and selection (Molina-Barahonaa et al., 2004). Hence, the addition of nutrients to MCD to produce PES provided additional nutrient for hydrocarbon degrading microorganisms which could be responsible for high degradation of the hydrocarbon contaminant in soil. The results of this study indicated that application of PES can enhance biodegradation of crude oil through increased bioavailability of petroleum hydrocarbons to soil microorganisms.Mohammad et al. (2010) reported similar finding.

$$Y = -0.0583t^2 + 0.5474t + 85.726 \quad (3.0)$$

$$Y = -0.2249t^2 + 5.2099t + 45.896 \quad (4.0)$$

Where Y is the PES/MCD efficiency (%) and t is the remediation time (days).

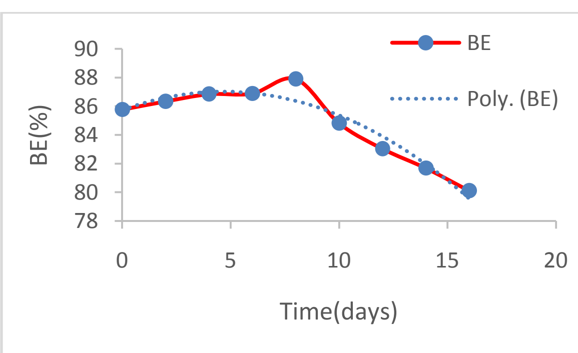

Fig. 13.0: Bio-stimulation efficiency of PES in soil

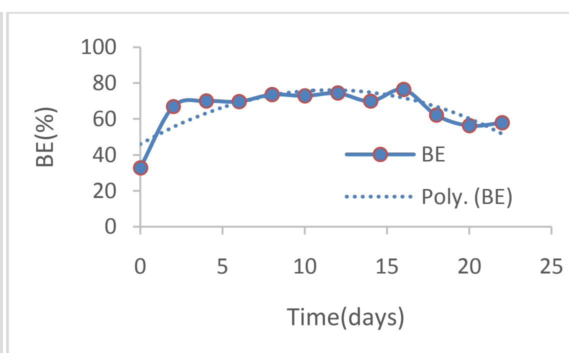

Fig. 14.0: Bio-stimulation efficiency of MCD in soil

4.0 Conclusion

This study demonstrated that bio-stimulation of biodegrading crude oil with PES enhanced degradation of crude oil under laboratory conditions. The TPH degradation in the crude oil contaminated soil was enhanced by bio-stimulation with nutrient present in the PES. PES contains significant amount of nutrient (potassium, nitrate, phosphate, sulphate and calcium) which help in enhancement of biodegradation of crude oil polluted soil by increasing the microbial activities of the indigenous biodegrading microbes. The % removal of TPH was found to increase as the concentration of PES (mg/l) increase. % TPH also decreased with increase in concentration (mg/l) of crude oil. Increase in depth of soil reduces the efficiency of TPH removal. The PES stimulated the biodegradation more than the MCD. Therefore, application of PES to stimulate the biodegradation of crude oil polluted soil can enhance the degradation of crude oil through increased bioavailability of petroleum hydrocarbon to soil microorganism.

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