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INVESTIGATION OF BIOSTIMULATION AND BIOAUGMENTATION FOR ENHANCED BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL

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ABSTRACT

Biostimulation using organic and inorganic biostimulants (poultry droppings and NPK fertiliser) and bioaugmentation using a mixed microbial consortium (Aspergillus niger and Pseudomonas aeruginosa) was evaluated for enhancing the bioremediation of a crude oil contaminated soil. The extent of remediation was evaluated by monitoring the pH of the soil, residual hydrocarbon content (RHC) and total microbial count (TMC) for 8 weeks. The pH of the samples was observed to increase during treatment albeit within the range of 6 – 7.6. The RHC decreased for all samples with the highest percentage reduction (99.88%) resulting for the sample containing both the biostimulant and the mixed microbial consortium. This result is significant when compared with the control which recorded a small percentage degradation (47.46 %). The TMC for most of the samples followed the typically observed TMC profile. Conclusively, the incorporation of biostimulation and bioaugmentation could be seen to have enhanced the bioremediation of the crude oil contaminated soil..

1. INTRODUCTION

The first automobile air conditioning system introduced by Packer in 1939 used a vapour compression refrigeration system (Tiwari and Parishwad, 2012). In the last 70 years, automobile air conditioning systems have undergone gradual and continual improvements in performance and efficiency as a result of improvements in the individual components (McLaughlin, 2005).

Over the years, due to poor safety consciousness of the oil industry, vandalization of oil pipe lines by and oil tanker's accidents, crude oil has spilled onto the environment, thus contaminating it, and making it unfit for agricultural practices. There is thus the need for remediation because crude oil spills may cause damage to the environment in many ways. Oil spill on land may lead to retardation of vegetation growth and cause soil infertility for a long period of time until natural processes re-establish stability (Onifade et al., 2007).

Crude oil contaminated soil can be reclaimed using several methods, one of which is bioremediation. Others include extraction with solvents and addition of chemical oxidizers. Bioremediation is the use of biological methods to restore contaminated land, especially the addition of bacteria and other organisms that consume or neutralize contaminants in the soil. It is a modern method, in which the natural ability of microorganism is employed, for the reduction of the concentration and/or toxicity of various chemical substances such as petroleum derivatives, aliphatic and aromatic hydrocarbons, industrial solvents and metals (Korda et al., 1997). Bioremediation was employed on a test basis on Alaskan beaches contaminated with oil released by the Exxon Valdez in 1989. Initial field tests revealed one brand of fertilizer that best stimulated bioremediation of the oil, and that fertilizer was then applied to portions of the oil-coated beaches. In those areas, the beaches appeared dramatically cleaner than in untreated areas, but the complexity of the chemical composition of crude oil makes determining the true effectiveness of the treatment very difficult. Due to the cheap nature of bioremediation, its reliability and efficacy, several researches have thus been conducted to determine its applicability, factors that facilitate it, as well as inhibiting factors. Obahiagbon et al. (2014) showed that the initial pH of the site, have effect on the bioremediation of a crude oil contaminated site. They also showed that Biodegradation was highly inhibited in the very alkaline (high pH values) and very acidic conditions (low pH values). Otokunefor and Obiukwu (2010) also showed that inorganic nutrients aid bioremediation. Microbial growth in the presence of urea was directly proportional to nutrient supplement concentration.

Hydrocarbon biodegradation in soil can be limited by many factors, such as microorganism type, nutrients, pH, temperature, moisture, oxygen, soil properties, and contaminant concentration (Bradi et al., 2000; Semple et al., 2001; Sabate et al., 2004; Ghazali et al., 2004; Walter et al., 2005; Atlas and Bartha, 2006). These researchers have concluded that the disappearance of crude oil from seawater could be accelerated by the addition of nutrients such as nitrogen or phosphorus or both. Recommendations have been advocated for the microbial seeding of oil spills, because bacteria and fungi are the only biological species which have the metabolic capability of utilizing petroleum carbon for cell synthesis (Jobson et al., 1974).

This study was centered on the bioremediation of crude oil contaminated soil by biostimulation with poultry droppings, urea, and NPK fertilizer, as well as augmentation with a mixed microbial consortium of *Aspergillus niger and Pseudomonas aeruginosa.* The efficacy of the process was determined by monitoring the pH of the soil, residual hydrocarbon content (RHC), and total microbial count, (TMC).

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2. MATERIALS AND METHODS

2.1. Sample Collection

Crude oil of specific gravity 0.9206 was obtained from the Shell Petroleum and Development Company (SPDC), Warri, Nigeria. *Aspergillus niger and Pseudomonas aeruginosa* were both obtained from the Microbiology laboratory of the University of Benin and used as bioaumentation agents.

2.2. Sample Preparation

The crude oil polluted soil was simulated in 6 cells. To each cell, 870 mL of crude oil was added with 4 kg of soil. To cell H_2, biostimulants (poultry droppings and NPK in the ratio of 1:1) were added. To cell H_3 biostimulants (poultry droppings and Urea in the ratio 1:1) were added. To cell H_4, biostimulants (poultry dropppings, urea and NPK were added in the ratio 2:1:1). To the cell H_5, mixed microbial culture of *Aspergillus niger and Pseudomonas aeruginosa* was added. To the cell H_6, a hybrid of biostimulation and bioaugmentation was applied, thus, the poultry droppings, NPK, Urea (in ratio 2:1:1) and the mixed microbial culture was added. The cell H_1, which served as the control contained just the contaminated soil. Bioremediation indication parameters (TMC, pH and RHC) were monitored during the period of bioremediation (8 weeks). Sampling was done weekly.

2.3. Analytical Method

The RHC of the soil was determined by shaking 5 g of the soil sample with 25 mL of n-hexane and the absorbance of the extract was determined at 450 nm. The RHC was then extrapolated with reference from the standard calibration curve obtained from the absorbance readings at varying concentrations. The pH of the samples were determined using Fisher Accruement pH meter. The TMC given in cfu/g was determined using a colony counting chamber.

3. RESULTS AND DISCUSSION

Figure 1 shows the variation of pH with the bioremediation time. As observed from the figure, the pH varied considerably with the bioremediation time, which is an indication of occurrence of biological processes, majorly biodegradation (Hatzikioseyian, 2010). The pH of all cells from H_1 through H_6 increased, which is an indication that there has been mineralization of the crude oil in to less toxic substances such carbon (IV) oxide and water. Similar observations were made by Amenaghawon et al. (2014) in their investigation of the impact of nutrients, aeration and agitation on the bioremediation of crude oil polluted water using a mixed microbial culture consisting of *Aspergillus niger and Pseudomonas aeruginosa.* The pH values obtained varied between 6 and 7.6, which falls within the optimum pH range of 5 through 9 as considered by the Department of Environmental Quality (1998) and also within the acceptable pH limit of 6 through 9, as stipulated by FEPA (1997).

Figure 2 gives the variation of RHC with the bioremediation time. The reduction in the RHC values is indicative of a reduction in the crude oil content of the soil samples as a result of the mineralization of the hydrocarbons by the microorganisms in to less toxic substances, thus indicating that bioremediation has occurred. (Otokunefor and Obiukwu, 2010). The reduction in RHC, as observed from the figure 2 above is generally high and this can be attributed to the biodegradability of the crude oil, due to the fact that it occurs naturally and thus made up of naturally occurring compounds such as Hydrocarbons. (Department of Environmental Quality, 1998). Biodegradation occurred in each cell, including the control (H_1). The reduction in the RHC in the control (7108.23 mg/Kg – 3734.69 mg/Kg) which is 47.46 %, can be attributed to the presence of indigenous microorganisms that were capable of mineralizing the contaminant (Obahiagbon et al., 2014; Amenaghawon et al., 2014). These indigenous microorganisms may be as a result of the type of soil (Loamy Soil) used as the remediating media. Amenaghawon et al (2014), recorded a significant reduction (44.1 %) in the RHC of their control. The RHC in the other cells from H_2 (oil + soil + poultry droppings + NPK), through H_6 (oil + soil + poultry droppings + NPK + Urea + mixed microbial culture) decreased more, compared to that of the Control (H_1), as observed from the figure 2 above. This is due to the presence of nutrients added to the contaminated media. Cell H_2 (oil + soil + poultry droppings + NPK), supplied sufficient nutrient (nitrogen, phosphorus and potassium) required by the microorganisms to survive and metabolize the contaminant. Also the poultry droppings contains some inherent microorganisms such as *histoplasma capsulatum* and as such serves as a bioaugmentant. This thus explains the observed higher RHC reduction compared to H_3.

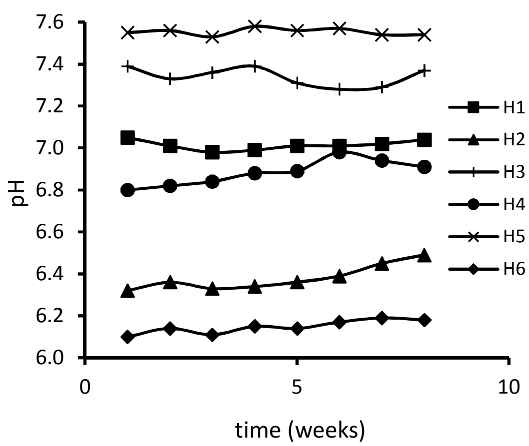

Figure 1: Variation of pH with bioremediation time

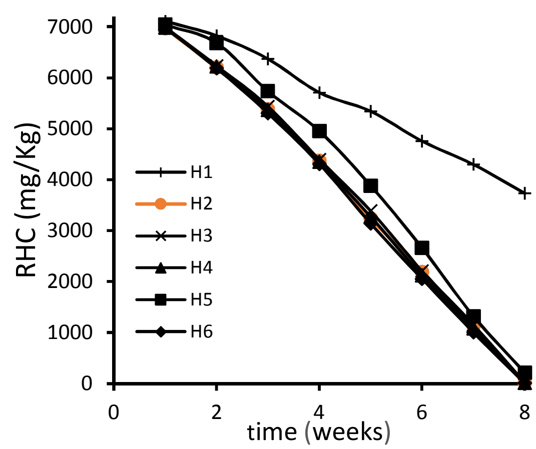

Figure 2: Variation of RHC with bioremediation time

Figure 3 shows the percentage degradation of the contaminant (crude oil) with the time during the course of bioremediation. In general, the percentage degradation for all cells increased with increase in bioremediation time. The high percentage degradation can be attributed to the fact that the crude oil is a naturally occurring compound. The success of Bioremediation depends on the chemical structure of the contaminant. (Neilson and Allard, 2008; Beek, 2001; Department of Environmental Quality, 1998). From Figure 3, Cell H_6 (oil + soil + poultry dropping + NPK +urea + mixed microbial culture) gave the highest percentage degradation (99.88 %), while that of the Control (cell H_1) gave the lowest percentage degradation (47.46 %). This can be attributed to the fact that cell H_6 employs a hybrid of biostimulation and bioaugmentation strategies and thus, the rate of biodegradation would be enhanced. Thus Biostimulation was improved by bioaugmentation (Bijay et al., 2012). The low percentage degradation compared to other cells, observed for the control can be attributed to the fact that it employed Natural Attenuation bioremediation strategy, which is the biodegradation of pollutants from a pollution site by the indigenous microorganisms present in the site. (Amenaghawon et al., 2014; Onifade et al., 2007; Fono et al., 2006; Hatzikioseyian, 2010). Crude oil contains several organic compounds (aliphatic and Aromatic hydrocarbons) which may not degrade at the same rate, thus there is need for mixed microbial culture (Department of Environmental Quality, 1998). Consequently, cell H_5 (oil + soil + mixed microbial culture) gave a percentage degradation of 96.92 at the end of the 8weeks as compared to the control (47.46). Favourable percentage degradation was also observed in the cells- H_2 (oil + soil + poultry droppings + NPK), H_3 (oil + soil + poultry droppings + urea), H_4 (oil + soil + poultry droppings + NPK + Urea), as well as H_5 (oil + soil + mixed microbial culture). Cell H_2 gave a large percentage degradation, (99.74 %) succeeding cell H_4 (99.81%). This indicates that biostimulation enhances natural attenuation. The percentage degradation of H_2 greatly varies from that of the control, indicating that the microorganisms present required the nutrients that were supplied by the bioaugmentants, as such, the N_2 content of the soil (without stimulating) was not sufficient to cater for the microorganisms present in the soil. Consequently, the need for biostimulation as obtained in cell H_2, which incorporated NPK and poultry droppings. Similar results were obtained by horom et al., 2010. They employed biostimulation using agricultural fertilizer (NPK 20: 10: 10) in order to enhance microbial degradation. The soil was artificially polluted with crude oil of about 1 % density and the fertilizer applied in 3 levels of 0, 1 and 2 tons/ha in 3 replicates. The results obtained indicated that the applied fertilizer increased the biodegradation. Cell H_4 (oil + soil + poultry droppings + NPK +urea) gave a higher percentage degradation (99.81 %) than cell H_2 (oil + soil + poultry droppings + NPK). This is due to the presence of Urea introduced, thus increasing the nutrient available to the microorganism. Cell H_3 (oil + soil + poultry droppings + Urea) gave a less percentage degradation (99.67%) compared to cell H_2, indicating that NPK is richer n nutrient concentration compared to Urea. Although Cell H_5 had a mixed microbial culture (*Aspergillus niger and Pseudomonas aeruginosa),* its percentage degradation was not higher than those obtained from biostimulation. This is because the nutrient present in the contaminated soil were limited and as such microorganisms had to compete for soil nutrient. Thus, bioaugmentation, although it favourable, is optimum when combined with biostimulation.

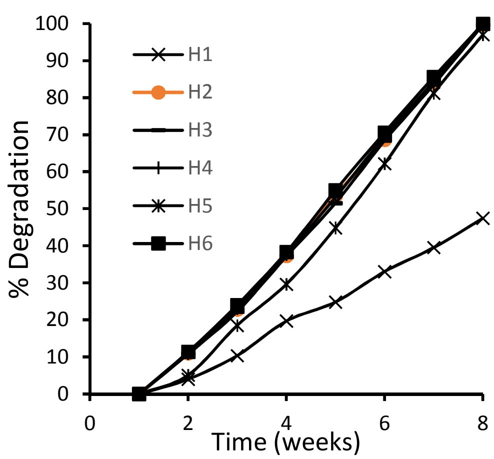

Figure 3: Percentage degradation of the crude oil with bioremediation time

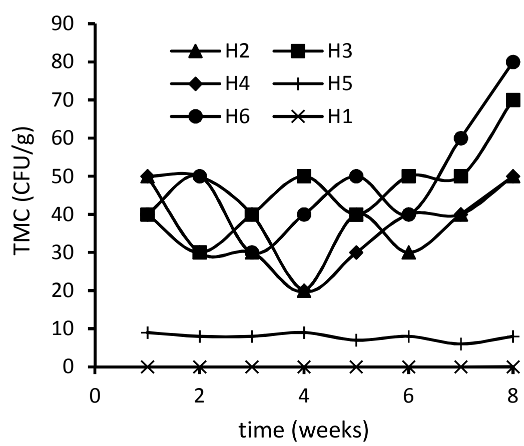

Figure 4: Variation of TMC with bioremediation time

Figure 4 shows the variation of TMC with time. The TMC expressed as the number of cells in 1 mL of a suspension is indicative of the concentration of microbial consortium present in the crude oil sample. Mixed microbial culture consisting of *Aspergillus niger and Pseudomonas aeruginosa* was used. This is because the Fungi- *Aspergillus niger,* consumes the nutrient, while the Bacteria (*Pseudomonas aeruginosa)* although consumes nutrient, can also synthesize nutrient. Thus there exist a symbiotic relationship between them. Cell H_6 indicated the highest increase in total microbial count (from 40 million through 80 million cfu/g) as observed from the graph. This might be as a result of the initial pH of the experimental set up, (pH of 6). The observations of Obahiagbon et al. (2014) supports this claim. They determined the optimum pH for microbial growth to be 6. The low microbial growth of H_5 can be attributed to competition between indigenous and exogenous microorganisms for the nutrients (Abdulsalam and Omale, 2009). Cell H_1 gave a TMC profile as typically observed. Initially, the microbial count reduced from week 1 through week 3, which indicates the lag phase, where the microorganisms get accustomed to the media. The reduction in population is as a result of the death of microorganism, resulting from the toxicity of the contaminant. Adams et al. (2015) suggested that the contaminants may have toxic effects on the present bacteria, when in high concentrations. Cell H_4 followed the profile as observed by the cell H_1, except that the microbial population reduced from week 1 through week 4. The TMC profiles for cells H_2, H_3, H_4 deviates a bit from the typically observed pattern.

4. CONCLUSION

The efficiency of bioremediation is optimal using a hybrid of biostimulation and bioaugmentation as observed by the results obtained for cell H_6 (hybrid of biostimulation and bioaugmentation) having the highest percentage degradation. (99.88 %). The cell H_6 also showed the highest increase in total microbial count from 40 million CFU/g through 80 million CFU/g. The pH of all cells progressively increased indicating that the contaminant were mineralized into less toxic substance.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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