Impact of Oxygen Diffusion on Indigenous Bacteria at 100cm Depth for Bioremediation of Hydrocarbon Contaminated Soils

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ABSTRACT

This research work examined the impacts of oxygen diffusion on indigenous Bacteria during bioremediation of Hydrocarbon contaminated soils at 100cm depth in the Niger Delta region of Southern Nigeria. An experimental investigation was carried out on the impacted soils by polluting three different soil samples (Sandy soil, Sandy loam and Clay soil) with Bonny Light Crude Oil. The reasons of polluting the soils with crude oil was to showcase a condition of major crude oil spill. Oxygen was diffused through each of the polluted soil samples in the reactors. Analysis of Total Petroleum Hydrocarbon at two week intervals for six weeks using Gas Chromatograph-Flame Ionization Detector (GC-FID) revealed that there was a decrease in concentration of Total Petroleum Hydrocarbon in the soils at the depth of 100cm. Also, the results indicated that there was an increase in population of indigenous Bacteria due to availability of sufficient oxygen at 100cm depth due to oxygen diffusion. The study showed that hydrocarbon concentration reduced by 71% for sandy soil, 75% for sandy loam and, 85% for clay soil at the end of the study period of six weeks.

Keywords: Bioremediation, Diffusion, Indigenous Bacteria and Pollution.

1. INTRODUCTION

The potential to degrade and utilize Petroleum hydrocarbons has been noticed in a number of bacteria, fungi and yeast. Bacteria are mostly used during soil bioremediation because they are distinguished by high frequency, fast growth and a wide spectrum of the utilized petroleum products (Wolicka *et al.*, 2009). Methylomicrobium has the potential of oxidizing halogenated hydrocarbon (Margesin & Schinner, 1999). Sorkhohet *et al.* (1992) reported that Bacillus spp are effective in biodegradation of petroleum contaminated soils and two major species are responsible for degrading of about 80-89% of the crude within 5 days at their optimum growth temperature of 60 °C. It was noticed that microbial response of Photobacterium phosphoreum increased remarkably while hydrocarbon degradation in contaminated soil was enhanced as a result of stimulation of microbial population size and activity from high rate of organic amendment (Liu *et al.*, 2009) Microbial communities in a contaminated soils is dominated by strains of bacteria that can survive toxicity and are able to make use of the contaminants for growth (Ijah & Antai 2003). As a response to bioremediation treatment, these populations may begin to actively degrade the pollutants and detoxify the soil, allowing other starving populations to increase their numbers, leading to an increase of bacterial community in the soils (Zucchi *et al.*, 2003). Biodegradation will occur in all types of soil, even though some may need additives or special care and equipment. Clay soils may need to be amended with bulking agents, in order to improve oxygen transport, also sandy soils may need to be amended with organic matter to improve the soil water holding capacity. The reasons for this may be low water holding capacity, low total organic carbon content,

and/or low surface area available for microbial growth in sandy soils (Nakles & Ray, 2002). The soil environment is the most dynamic site of interactions in nature (Scherret *et al.*, 2007). Microbial community carries out the majority of decomposition processes in the soil and are irreplaceable in the transformation and degradation of synthetic organic compound and natural waste material (Obire & Nwanbete, 2001). Soil permeability, pore and fractures in the rock are critical factors controlling the movement of water from the surface into the underlying layers (Atlas & Philips, 2005). The permeability of soil to air and water is a function of soil texture. Fine-textured soils like clays have low permeability, which prevents bio-vented oxygen and nutrients from dispersing throughout the soil. Fine textured soils are slow to drain from water-saturated soil condition, thus preventing oxygen from reaching soil microbes throughout the contaminated area (USEPA, 2006).

Clay minerals increase the rate of bacteria growth and influence hydrocarbon degradation (Challan & Tazaki, 2005). Soils with sandy texture and low organic carbon are known for poor microbial proliferation and diversity brings about lower degradation rates as compared to clay loam and loam (Hejazi Husain, 2004). The low amount of degraders in the sand fraction of contaminated soils is mutually related to a higher carbon/nitrogen ratio and lower internal surface (Amella *et al.*, 2001). Biodegradation is active and effective in an environment with adequate supply of oxygen. Only on rare occasion does biodegradation take place in anoxic environments. Biodegrading bacteria needs oxygen to break down petroleum hydrocarbon. Oxygen as a major factor to bioremediation processes, its delivery is important to the success or failure of bioremediation processes. In soils the oxygen content depends on the microbial activity, soil texture, water content and soil depth. Also, mineralization of hydrocarbon from soil was severally limited when the oxygen content was below 10% (Vasudevan & Rajaram, 2001). The importance of oxygen comes from the participation of oxygenases and molecular oxygen involved in the major degradation pathways for the hydrocarbons.

2. MATERIALS AND METHODS.

SOIL SAMPLING

The Soil samples for this study were collected from Kolo creek in Bayelsa State, Oshie oil field, Ahoada west and Abuaodua in Rivers state. It was collected using hand soil auger. The soil samples collected were bulked together and put in a well labelled Polyethylene bags and glass bottles, sealed with aluminum foil, for Total Petroleum Hydrocarbon (TPH) analysis.

EXPERIMENTAL DESIGN OF OXYGEN DIFFUSION IN THE SOIL.

Fifty kilogram (50kg) of soil samples were weighed into three bowls and contaminated with 5000mls (5litre) of Bonny light crude oil each measured using 1000mls measuring cylinder five times by using standard pollution volume of 100mls of crude oil to 1kg of soil. This was done after collection of soil samples for determination of physiochemical properties. The objective was to simulate condition of major crude oil spill. Each of the mixture was properly mixed to ensure uniform concentration of the crude oil in the soil samples. It was left for three days to settle without any disturbance. The treatment of the soil commenced after three days by application and mixing of 340g of 27:13:13 NPK fertilizer with the soil samples B and C, while sample A had no fertilizer application because it served as control unit or sample for the process. Thereafter, the three soil samples were transferred into three separate batch reactors labelled A, B, and C, of 1 meter depth and $4 \times 10^{-2} \text{m}^3$ volume, with application of 200mls of water every two days for 6 weeks to effect quick compartment and settlement of the soil samples. Soils in reactor C had a PVC pipe which transferred oxygen gas from the gas cylinder into soils in reactor C. The PVC pipe had perforation of 0.42mm in diameter of

four perforation per 10 cm intervals along the length of the Pipe. The purpose of the perforation was to allow the transfer of oxygen gas from the PVC pipe into the soil samples. A gas flow meter was fixed on the flow line into the reactors to measure the volume of oxygen transferred into the system. 14m³ of oxygen was delivered into soils in reactor C at an interval of 10minutes for 48hours and left to diffuse into the soil for a period of 42 days. Soil samples were collected from the reactors at intervals of 2, 4 and 6 weeks for Total Petroleum Hydrocarbon(TPH) and hydrocarbon utilizing bacteria analysis (Umeda *et al.*, 2017)

Determination of Total Petroleum Hydrocarbon.

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 as reported by Risdon *et al.* (2008).

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 10¹ diluted soil sample was placed on it and it was spread using a glass rod spreader to dryness on the plate. This was incubated in an incubator at 37⁰C for 24hours and the counting of the bacteria was made on the plate after the bacteria have shown growth. The bacteria that did not grow after 24hours were further allowed in the incubator for another 24hours and readings were made on them. The total bacteria count was made and recorded (Barrow & Feltham, 2003).

Cultivation of Hydrocarbon Utilizing Bacteria

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 according to Okpokwasili&Odokoma (1990). The plates were dried and 0.1ml of the 10¹ diluted soil samples were placed on the dried plates. The samples were spread on the minerals salt agar plate using the glass rod spreader to dryness. A crude oil (Bonny light) soaked 90mmØ 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 test were carried out to identify the bacteria (Okpokwasili&Odokoma, 1990).

Isolation and Identification of Bacteria

Cultural bacteria were prepared by aseptically streaking representative colonies of different cultural types, which appeared on the culture plates, onto nutrient agar-plates and incubates at 28 ⁰C for 24 hours. The nutrient agar plates were stored in a refrigerator and served as pure stock culture for subsequent characterization and identification tests. Standard characterization tests (such as grain staining, motility, oxidase test, and Catalase test) were performed. The pure cultures were identified on the basis of its cultural, morphological and physiological characteristics (Barrow and Feltham, 2003).

Degree of Degradation of Hydrocarbon

Degree of degradation of hydrocarbon was done by calculation, using Equation (1)

$$\% D = \frac{TPH_i - TPH_f}{TPH_i} \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

Table 1. Particle Size Distribution and Classification

Soil Type	Sandy (%)	Silt (%)	Clay (%)	Porosity	Bulk Density(g/cm ³)	Diffusion Coefficient(cm ² /s)
Sandy Soil	88.4	2.94	8.66	0.521	1.269	0.007
Sandy loam soils	78.44	6.80	10	0.547	1.201	0.004
Clay soil	8.66	14.76	60	0.463	1.424	0.004

Table 1 shows Particle Size Distribution (PSD) and soil classification of the three different soils used in the study. The results in the Table indicated that, the soils analyzed were Sandy soil with 88.4% Sand, 2.94% Silt and 8.68% Clay, followed by Sandy loam soil with 78.44% sand, 6.80% silt and 14.76% Clay and finally Clay soils with 30% Sandy, 10% Silt and 60% Clay. Also, the Table showed the bulk density and porosity of the soils as 1.269g/cm³ and 0.521 for Sandy soil, 1.201 g/cm³ and 0.547 for Sandy loam soils and 1.424 g/cm³ and 0.463 for Clay soils.

Variation of Hydrocarbon Utilizing Bacteria with Time

Figure 1. *Variation of Hydrocarbon Utilizing Bacteria with Time, Sandy Soils* **Figure 2.** *Variation of Hydrocarbon Utilizing Bacteria with Time, Sandy Loam*

Figure 3. Variation of Hydrocarbon Utilizing Bacteria with Time, Clay Soils

The analysis of the initial conditions of Sandy soil samples before contamination with crude oil showed Total Heterotrophic Bacteria count as 2.88×10^3 cfu/ml, which include *Bacillus* species, *Pseudomonas* species, *Mycobacterium* species, and *Arthrobacter* species. The Bacteria count reduced from 2.88×10^3 cfu/ml to 2.28×10^3 cfu/ml, after three days of pollution of the soil samples with crude oil. Also, the results of Initial conditions of Sandy loamy soils indicated Heterotrophic Bacteria count of 2.70×10^3 cfu/ml before pollution with crude oil which identified the presence of *Bacillus* species, *Pseudomonas* species, and *Mycobacterium* species in Sandy loam soil. The Bacteria count result gave a decrease of 2.01×10^3 cfu/ml of bacteria after three days of contamination of the soil with crude oil. Further results of initial conditions of Clay soil have Total Heterotrophic Bacteria count of 3.98×10^3 cfu/ml before pollution with crude oil. The bacteria species identified in clay soils before pollution with crude oil were *Pseudomonas* species, *Arthrobacter* species and *Bacillus* species. The bacteria count reduced to 1.72×10^3 cfu/ml after contamination with crude oil. Analysis and identification of different species of the bacteria in Sandy soil, Sandy loam and Clay soils indicated that *Bacillus* Species were the only surviving hydrocarbon utilizing bacteria in the petroleum contaminated soil samples at 100cm depth. This was possible because they have spores that protected their inner cells from been destroyed by the crude oil pollution at 100 cm depth. In Figures 1, 2 and 3, soils in reactors A and B had a decrease in bacteria count at 100cm depth, but the soils in reactor C recorded a significant increase in bacteria count for the entire period of bioremediation. The decrease in bacteria count in soils in reactors A and B indicated that there was insufficient oxygen at the 100cm depth for microbial growth in population, but the significant increase in microbial population in soils in reactor C was because of the presence of sufficient oxygen at 100cm depth by oxygen diffusion through the hydrocarbon contaminated soils.

The effect of Hydrocarbon Utilizing Bacteria on Total Petroleum Hydrocarbon with Time.

Figures 4, 5 and 6 show the effect of Hydrocarbon Utilizing Bacteria on Total Petroleum Hydrocarbon with Time. The graphs showed that the concentration of Total Petroleum Hydrocarbon decreased as the population of the Hydrocarbon Utilizing Bacteria increased with Time. The increase in population of the microorganisms in the soils at 100cm depth were due to sufficient oxygen by oxygen diffusion. The increase in population of the microorganism gave rise to high demand and utilization of the hydrocarbons by the bacteria as source of carbon for energy thereby reducing the concentration of the Total Petroleum hydrocarbons in the impacted soils.

Figure 4. Variation of HUB and TPH with Time

Figure 5. Variation of HUB and TPH with Time

Figure 6. Variation of HUB and TPH with Time

4. CONCLUSION.

Oxygen diffusion through petroleum contaminated soils enhanced increase in population of hydrocarbon utilizing bacteria which promotes degradation of hydrocarbons in petroleum contaminated soils at 100cm depth. Also *Bacillus* Species was the only Bacteria Species that survived at the depth of 100cm for bio degradation of petroleum contaminated soils. Hence, this method is considered effective in treatment of polluted soil at higher depth in the soils.

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