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# Kinetic Studies of Bioremediation of Hydrocarbon Contaminated Groundwater

**Medjor O.W[1], Egharevba F.[2], Akpoveta O. V.[2], Ize-lyamu O.K.[2] and Jatto E.O[2]**
[1]Department of Chemistry, Taraba State University, Jalingo, NIGERIA
[2]Department of Chemistry, Ambrose Alli University, Ekpoma, NIGERIA

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## Abstract

*Bioremediation experiments were conducted at ambient temperature of 28-32$^0$C and pH 5.6-8.9 to investigate the effectiveness of the process in the clean-up of groundwater contaminated by diesel. Polluted groundwater samples were simulated in the laboratory by contaminating 900ml of groundwater sample with 100ml of diesel to achieve 10% pollution in two different plastic microcosms. Two tests series were performed for a 1008-hour (42-day) residence time. In the first test, polluted groundwater sample was taken in a plastic microcosm without organic amendment (mixed-culture of pig, cow and poultry wastes). In the second test, organic amendment was added to the polluted groundwater sample in the second plastic microcosm and the bioremediation process in both cases allowed to proceed. Microbiological and TPH analyses were carried out weekly for six weeks on the second microcosm and at the sixth week for the first microcosm which acted as control. The indices of biodegradation monitored included total changes in: total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB), total fungal (TF) counts and changes in total petroleum hydrocarbon (TPH).The response of the indigenous microbes (heterotrophs, hydrocarbon utilizers and fungal) was positive in the second microcosm where biodegradation occurred as a result of the microbial activities. No appreciable biodegradation occurred in the control microcosm, except for about 3% loss of total petroleum hydrocarbon due to evaporation. 91.53% removal efficiency for total petroleum hydrocarbons was obtained in the first microcosm at the end of the sixth week. Bioremediation of groundwater polluted with diesel is a first order reaction with rate constant of 0.002hour$^{-1}$ and half-life ($t_{1/2}$) of 346.5 hours. The overall assessments of the quality of the contaminated water samples after remediation were close match to the unpolluted water sample with some selected physicochemical parameters (pH, DO, $BOD_5$, and salinity as chloride) within the WHO standard for surface/underground water while COD was far above limits recommended by W.H.O.*

**Keyword:** Groundwater, bioremediation, organic amendment, total petroleum hydrocarbon (TPH), removal efficiency.

## Introduction

The world today is very much dependent on crude oil, either to fuel the vast majority of its mechanized transportation equipment or as the primary feedstock for many of the petro-chemical industries. In the year 2003, crude oil production volumes surpassed 82.3 million barrels per day and this volume is estimated to increase to 94.3 million barrels per day in 2010 and 101.6 million barrels per day in 2015[1]. Oil or petroleum-hydrocarbons, therefore, represent high-volume global materials[2]. Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which possesses a measurable toxicity towards living systems[3].

According to Gutnick and Rosenberg as cited by[4], the increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment. Dubbed as the bloodline of modern civilization, petroleum-hydrocarbon compositions vary greatly in its complex mixture of hydrocarbons and other organic and inorganic compounds, which contribute to the diversity in its physical properties[5]. Crude oil and its petroleum derivatives also contain heavy metals. The world is facing a prospect of water shortages caused by population growth, uneven supplies of water, pollution and other factors.

The United Nations (UN) predicts that water shortages could retard the economic growth of some countries and lead to food shortages and possibly, to international conflicts[6]. Groundwater and rivers constitute the main sources of water supplies that humans use for drinking, cooking, cleaning, industry and agriculture. These several sources of water supplies are polluted by natural geological sources, pesticides, industrial discharged from various processing industries and oil spillage during oil exploration and exploitations or accidental discharge. Organic substances from oil spillage and petroleum products disposal into water

bodies significantly contaminate and degrade them and could possibly elevate the concentration levels of heavy metals. Heavy metals are persistent and can easily enter food chain and accumulate until they reach toxic levels. Traces of heavy metals such as Hg, Cd, Pb, Co, Mn, Cu, Fe and Cr above stipulated levels are toxic to aquatic ecosystem and human[7,8]. Incessant oil spills are known to have caused severe damage to aquatic and terrestrial environment.

Crude petroleum, as well as many products derived from them, is shipped from oil-producing locations to oil-consuming locations in ocean-going vessels having huge capacities. Accidents in which the hulls of such vessels are breached and their contents spilled can cause serious damage to the environment. Unfortunately, such accidents are not at all uncommon. Land and offshore oil wells also can be a source of oil spills into ocean waters.

Oil spills from such accidents may quickly spread over many square miles of water surface, for example, in the Niger-Delta region of Nigeria where there is increasingly oil exploration and exploitation work. Incessant vandalization of pipelines carrying petroleum and its products, massive destruction of oil facilities by irked youths, oil bunkering, industrial and domestic accidents which result in oil spillage as well as petroleum products deposits into existing water bodies have become the order of the day. An environmentally sustainable society satisfies the basic needs of its people without depleting or degrading its natural resources and thereby not preventing current and future generations of humans and other species from meeting their basic needs.

## Material and Methods

**Sampling Method:** Underground water samples were obtained in pre-sterilized 10 litre containers from the tap of chemistry laboratory, Petroleum Training Institute (P.T.I.), Warri, Delta State in southern Nigeria, using the grab sampling method. These samples were immediately taken to the microbiology laboratory within the Institute for bioremediation experiments.

**Source of Nutrients:** The organic supplement (amendment) used in this work was mixed dung of cow, pig and poultry thoroughly mixed in 1: 1: 1 ratio.

**Preparation of Samples:** Polluted groundwater samples were simulated in the laboratory by contaminating 900ml of groundwater sample with 100ml of diesel to achieve 10% pollution in two different plastic microcosms. 25g of the organic amendment was added as a source of microorganisms to one of the microcosms while the second microcosm without the amendment acted as control.

**Bioremediation Experiment:** This experiment was set up to assess the ultimate bioremediation of hydrocarbons in water samples by indigenous microorganisms in mixed dung of cow, pig and poultry. The indices of biodegradation are total changes in: total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB), total fungal (TF) counts and changes in total petroleum hydrocarbon (TPH).

**Microbiological Analysis:** In order to isolate and enumerate both heterotrophic and hydrocarbon utilizing bacteria, bacteria enrichment processes using modified mineral salt medium of[9] and the spread plate technique as described by[10] were used. For isolation and enumeration of fungal isolates, Sabraund dextrose agar was used. Fungal isolates were identified using the method of[11].

**Total Petroleum Hydrocarbon (TPH) ANALYSIS:** TPH extraction and analysis were carried out following the laboratory manual adopted by[12]. A standard calibration curve was first constructed. A standard concentration of 1000ppm of diesel in hexane was prepared as standard stock. Working standards of 0, 10, 20, 40, 60, 80 and 100 ppm were prepared from the standard stock. At each time of 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 (2007 Model) at a wavelength of 350nm.

## Results and Discussion

The initial population of the total heterotrophic bacteria (THB) in the diesel microcosms was $41 \times 10^5$ cfu/ml. After 168hours, the population of the total heterotrophic bacteria had dropped to $6.3 \times 10^5$ cfu/ml as seen in table 1.This account for 15.37 % decrease in population of the total heterotrophic bacteria in the medium. When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, the microbes are likely to have no prior exposure to the contaminant in their natural environment and therefore may die or cause inhibition. It is also possible that the microorganisms have been injured and require time for the cells to retool, replicate their DNA, and begin to increase in mass, finally divide and eventually recover[13]. To adapt to their new environment, enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume[14]. The heterotrophic bacteria experienced an unbalanced growth being transferred from the rich-amendment into the nutritionally poor diesel-water-medium. During the exponential or log phase, a peak growth $9.7 \times 10^5$ cfu/ml after 336 was achieved. This represented an exponential growth rate of 153.97% for the heterotrophs after the lag phase. The death phase set in immediately after the log phase, the stationary phase being absent because bioremediation process occurred in an open system. The similar trends of the lag, log and death phases exhibited by

the total heterotrophic bacteria were observed by the total hydrocarbon utilizing bacteria and fungal population. The initial population of the total hydrocarbon utilizing bacteria (THUB) was $2.8 \times 10^4$ cfu/ml. After the first 168hours, the population of the THUB had dropped to $1.3 \times 10^4$ cfu/ml and accounted for 4.64 % decrease in population of the total hydrocarbon utilizing bacteria. The growth peak for the total hydrocarbon utilizing bacteria (THUB) was $5.7 \times 10^4$ cfu/ml (438.46%) after 336 hours. The initial population of fungal in the polluted water sample in the second microcosm was $1.13 \times 10^4$ cfu/ml. There was no drop in the initial fungal population. This is generally contrary to expectations as when microorganisms are introduced into fresh culture medium, usually no immediate increase in cell occurs (lag phrase).The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. A possible explanation for the initial observed growth may be that the microorganisms from the organic amendment (mixed-dung of poultry, cow and pig) are young with vigorously growing exponential phase therefore the lag was short or absent. The fungal population in the first 168 hours grew better than the bacteria population. This is in conformity with the study done by[15], that fungi are more tolerant of acidic conditions. The growth continued exponentially until a peak growth of $4.8 \times 10^4$ cfu/ml (424.78%) after 504 hours was attained. There was no significant difference (at $p<0.05$) in the means of the microbial population used in the study.

The population of the microbial organisms used in the study is shown on table 1 and their growth curves represented in figure 1.

**Table-1**
**Microbial population used in the bioremediation experiment**

| Time (hours) | THB $\times 10^5$Cfu/ml | Cell density (Log Cfu/ml) | THUB $\times 10^4$Cfu/ml | Cell density (Log Cfu/ml) | FUNGAL $\times 10^4$Cfu/ml | Cell density (Log Cfu/ml) |
|---|---|---|---|---|---|---|
| 0.00 | 41.00 | 6.61 | 2.80 | 4.45 | 1.13 | 4.05 |
| 168.00 | 6.30 | 5.80 | 1.30 | 4.11 | 1.80 | 4.26 |
| 336.00 | 9.70 | 5.99 | 5.70 | 4.76 | 3.40 | 4.53 |
| 504.00 | 4.70 | 5.67 | 4.2 | 4.62 | 4.80 | 4.68 |
| 672.00 | 3.30 | 5.52 | 3.40 | 4.53 | 4.30 | 4.63 |
| 842.00 | 2.60 | 5.41 | 5.10 | 4.71 | 2.90 | 4.46 |
| 1008.00 | 3.10 | 5.49 | 2.50 | 4.40 | 2.10 | 4.32 |

Source: Laboratory Analysis 2011. Key: THB=total heterotrophic bacteria; THUB=total hydrocarbon utilizing bacteria

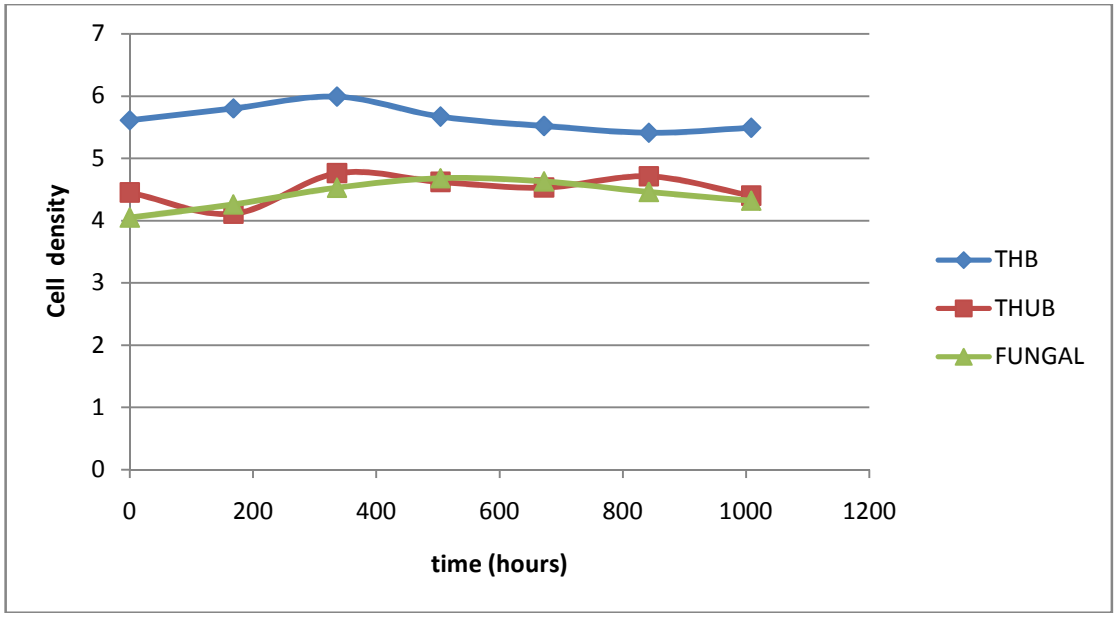


**Figure-1**
**Profile of Microbial Population Used in Bioremediation**

Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. The temperature readings of the experiments were in the mesophilic range of 28–32$^{o}$C, which is the ambient temperature in Nigeria[16]. Low temperature will affect microbial growth and propagation, and under normal circumstances, rates of degradation decrease accordingly[17]. This is a result of primarily decrease in the rates of enzymatic activity.

The optimum temperature is typically in the range of 30 to 40$^{o}$C. At temperature above this norm, enzymatic activities are inhibited as proteins denature[18].

There was a significant difference statistically in the pH throughout the experiment at $p<0.05$. It implies that bioremediation process is pH dependent. The pH value showed a sharp depression at 5.6 as seen in figure 2. The simultaneous production and accumulation of acidic metabolic products particularly during the first week of the experiments may have accounted for the decrease in the pH which may be responsible for the decrease in the bacterial population. This is consistent with the findings of[19,20]. The pH in the third week through the sixth week was within the range of 8.5–8.7 indicating enhanced microbial activity. Similar pH values for borehole water have been reported by the work done by[21].

**Table-2**
**Variation of mean pH values with time during bioremediation process**

| Time (hours) | pH |
|---|---|
| 0.00 | 8.0 |
| 168.00 | 5.6 |
| 336.00 | 7.9 |
| 504.00 | 8.9 |
| 672.00 | 8.7 |
| 842.00 | 8.6 |
| 1008.00 | 8.6 |

The order of the bioremediation reaction was investigated and results are shown in table 3and represented in figures 3 and 4. Once degradation of a chemical (diesel) commences, the amount disappearing with time and the shape of the disappearance curve is a function of the compound in question, its concentration, the organisms responsible and a variety of environmental factors. In the first order reactions (unimolecular reactions), a plot of ln (TPH) against time (t) is a straight line and the slope gives the value of the rate constant.

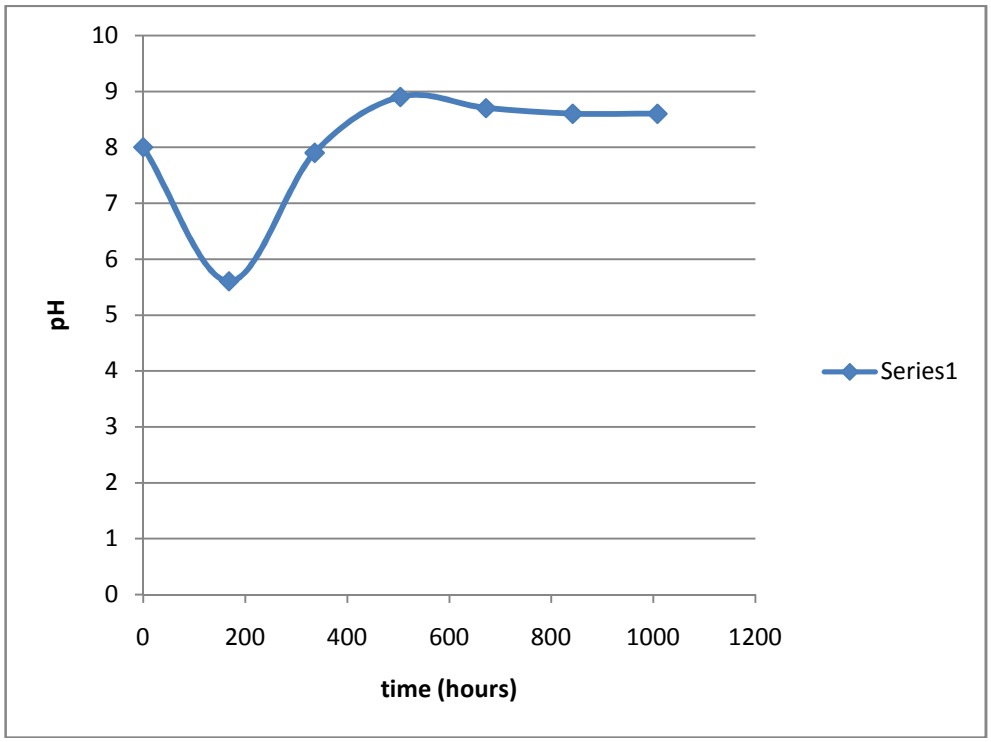


**Figure- 2**
**Variation of pH with time during bioremediation process**

The regression coefficients ($R^2$) obtained from the linear plot was 0.891. This suggests very strong relationship between the rates of biodegradation of TPH with time as seen in figure 4. It was obvious from the plot in figure 3 that the TPH concentration decreased with time as a result of the growth of microbes in the broth. Figure 3 showed an exponential curve for the bioremediation process. The exponential curve obtained in figure 3 in conjunction with the graph in figure 4 graphically satisfied the conditions for first order reaction, with a rate constant of 0.002hours and half-life of 346.5 hours. Bioremediation efficiency of 91.53% was achieved as seen in table 3. This suggests that the bioremediation process was very efficient and effective in the clean-up of groundwater contaminated with diesel.

**Table – 3**
**Data for kinetic Studies of Bioremediation**

| TIME/ HOURS | TPH LEFT | ln TPH | % REMOVAL |
|---|---|---|---|
| 0.00 | 5201.59 | 8.56 | 0 |
| 168.00 | 1623.04 | 7.39 | 68.8 |
| 336.00 | 1355.35 | 7.21 | 73.9 |
| 504.00 | 1115.55 | 7.02 | 78.55 |
| 672.00 | 917.93 | 6.82 | 82.35 |
| 840.00 | 631.5 | 6.45 | 87.86 |
| 1008.00 | 440.53 | 6.09 | 91.53 |

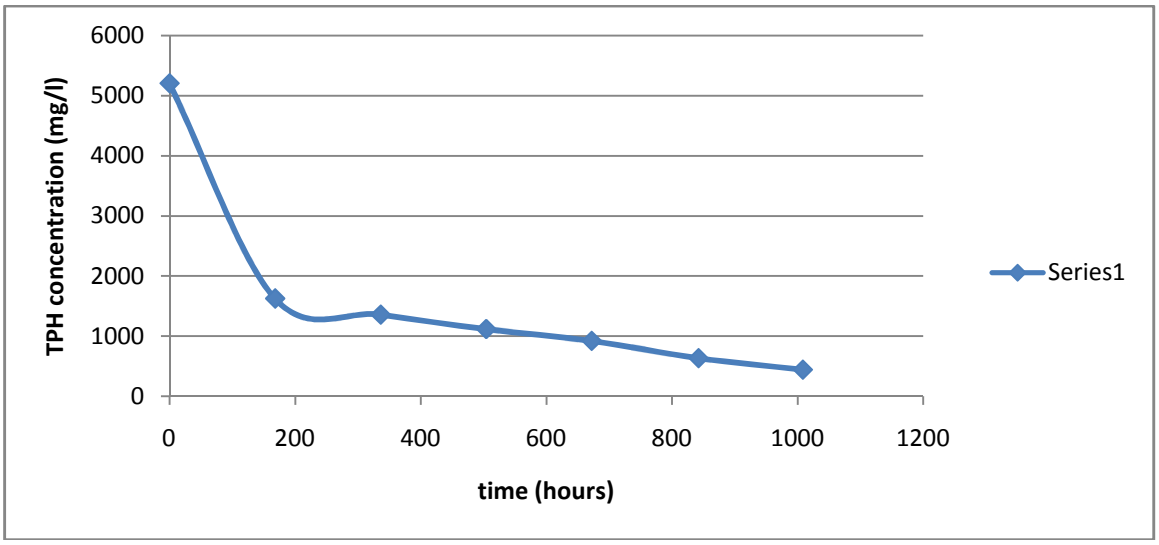


**Figure – 3**
**First order graph showing concentration of TPH as diesel against time for the bioremediation process**

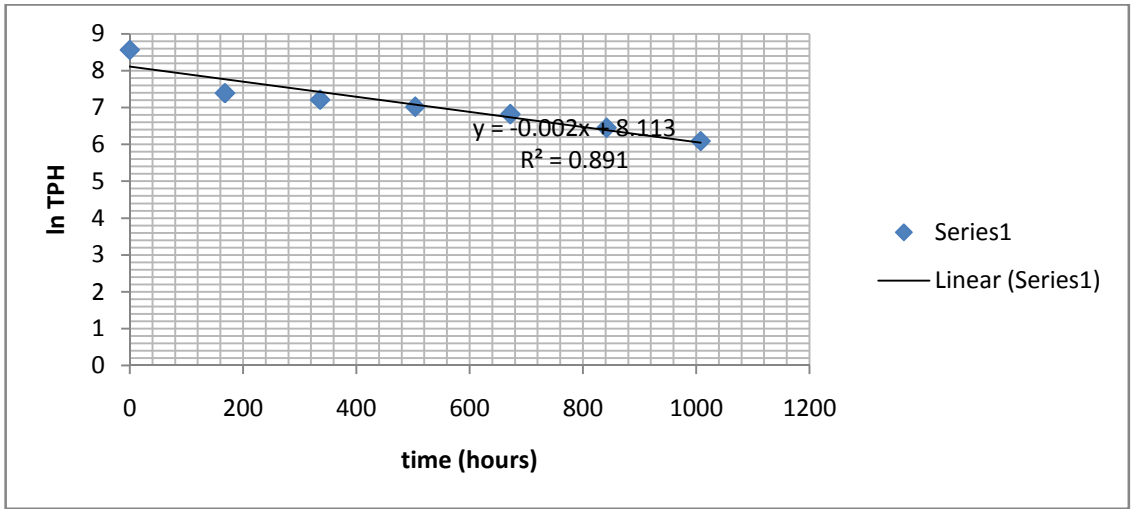


**Figure – 4**
**First order graph showing ln TPH as diesel against time for the bioremediation process**

**Table – 4**
**Physicochemical Properties of remediated sample and unpolluted groundwater sample**

| Parameters | pH | Dissolved oxygen (DO) mg/l | Biochemical oxygen demand ($BOD_5$) mg/l | Chloride ($Cl^-$) mg/l | Chemical oxygen demand (COD) mg/l |
|---|---|---|---|---|---|
| S1 | 7.30 | 3.60 | 1.80 | 71.20 | 12.2 |
| FI | 8.50 | 2.90 | 2.10 | 248.00 | 610.00 |

Source: Lab. Analysis 2011; S1 = unpolluted groundwater sample, FI = remediated sample

The increase in microbial population during the bioremediation process except for the control microcosm was an indication of their ability to utilize petroleum hydrocarbons as carbon and energy sources. According to[22], high population sizes suggest increase in hydrocarbon assimilation. According to[23] as cited by[20], the relative significance of bacterial to fungal population in hydrocarbon degradation has been an issue of controversy. In agitated enrichments, bacteria tend to have more predominant role, while some scholars suggest that fungi can be significant or even predominant in undisturbed surface slicks on land surface and water.

This controversy is also very evident in this study as it is difficult to clearly ascertain whether bacteria or fungi played the predominant role during the bioremediation process. Many microorganisms have been found to have the ability to utilize hydrocarbons as sole source of energy and carbon and such microorganisms are widely distributed in nature.

Oxygen is the first electron acceptor available for biotic transformations that is quickly consumed. The dissolved oxygen in the diesel microcosm was 2.9mg/l. The presence of dissolved oxygen is an indicator of electron acceptor availability. The initial dissolved oxygen in the water sample was 3.6mg/l. The drop in the initial dissolved oxygen indicated the occurrence of chemical, physical and biochemical activities of microbes. High oxygen (>2mg/l) shows aerobic conditions and oxygen will be the preferred electron acceptor until depleted[24].

The initial Biochemical Oxygen Demand ($BOD_5$) value of the water sample was 1.80mg/l. The $BOD_5$ values for the water sample contaminated with diesel in the second microcosm at the end of six weeks residence time was 2.10mg/l. This increase in the $BOD_5$ values suggest increased microbial biodegradation of the contaminant. Chemical Oxygen Demand (COD) provides a measure of the oxygen equivalent of that portion of the organic matter in a water sample that is susceptible to oxidation under test condition[25].

The initial COD for the water sample was 8.8mg/l. The COD values for the groundwater sample contaminated with diesel in the second microcosm at the end of 6 weeks residence time was 1.80mg/l. The initial low value of the COD (8.8 mg/l) in the unpolluted groundwater sample indicated the presence of small amount of organic matter. The high values of COD obtained in water sample contaminated with diesel in the second microcosm may be as a result of heavy organic load from the mixed-waste used as amendment.

Chlorine gas is highly toxic but chloride ions are essential for life[26]. Chloride occurs in all natural waters in varying concentrations. Concentration is usually greater in groundwater than surface water especially if salt deposits are in the area. Chloride in small concentrations are not harmful to humans in drinking water, and with some adaptation, the human body can tolerate water with as much as 200 mg/l chloride ion. However, above a concentration of 250 mg/l chloride, the water may taste salty[27].

The initial chloride ion concentration in the water sample was 71.2mg/l. The chloride ion concentration for the diesel medium was 248mg/l . These concentrations are within the W.H.O recommended standard for chloride ions in drinking water (100-250mg/l).

## Conclusion

The findings of this study show that bioremediation is a very promising and efficient technology in the clean-up of underground water contaminated with diesel. This is evident in the high removal efficiency obtained from the study. The remediation process was found to follow first order kinetics with a high degree and rate of remediation achieved within six weeks. The overall assessments of the quality of the contaminated water samples after remediation in terms of pH, DO, BOD, COD and chloride were close match to the initial water sample with most of the physicochemical parameters within the WHO standard for surface/underground water, except for COD which was far above the W.H.O. standard for surface/underground water limits. Therefore, bioremediation is highly recommended for the clean-up of water bodies contaminated with automobile gas oil (diesel). The method is cheap as microorganisms responsible for biodegradation are present in their millions in the waste of cow, pig and poultry which are easily obtainable. Major disadvantages of bioremediation include: time consuming and build-up of green house gases $CO_2$ and $NH_3$ as a result of enzymatic-transformation of the hydrocarbon and hence possibly contributing to global warming. The application of bioremediation must therefore be applied wisely with a word of caution.

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