Application of Aeration in the Bioremediation of Petroleum Hydrocarbon Polluted Water

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Abstract
Remediation of petroleum hydrocarbon polluted water was investigated through the application of aeration and aeration plus bioaugmentation. The experimental setup was maintained for a duration of 28 days, and monitored at weekly intervals for Total heterotrophic bacterial population (THB) and Hydrocarbon utilizing bacterial population (HUB). Phosphate, nitrate, and total hydrocarbon concentration (THC) was determined at the beginning and end of the experimental period. At the end of the study, there was a general decrease in the physicochemical parameters monitored; reduction of THC of about 52.6 % was obtained in the setup where only aeration was applied, while about 53.3 % reduction in THC was obtained in the setup where aeration plus bioaugmentation was applied. There was minimal difference between the two methods, and it is thus recommended that it would be economical to use aeration alone in the remediation of crude oil polluted aquatic environment. The bacteria isolated at the end of the study from the experimental setup include Vibrio sp., Bacillus sp., Pseudomonas sp., Neisseria sp., Micrococcus sp., and Staphylococcus sp.

Key words: *Remediation, Petroleum hydrocarbon polluted water, Aeration, Bioaugmentation, Pseudomonas species.*

Introduction

The presence of large quantities of petroleum hydrocarbons in an environment as a result of accidental discharge, indiscriminate disposal of waste generated from petroleum products, artisanal refining activities, or vandalism, renders the environment unfit for its natural functioning and purpose. The environment becomes unfit for use as a result of the negative changes created in the structure of the abiotic components, and toxic effects on the flora and fauna (Jenkins *et al*., 1978; Ojimba, 2012; Oyem and Oyem, 2013). The contaminated environment will also impact negatively on human health. For instance, the presence of benzene, a known carcinogen, has been observed in the groundwater of communities impacted with petroleum hydrocarbons (UNEP, 2011). Drinking of such impacted water will thus lead to deterioration in the health of the human population living in such communities. Environments contaminated with petroleum hydrocarbons thus need to be returned to their original state if the environments are to become useful again for its natural purposes.

There are a number of technologies for dealing with oil pollution both at sea and on shorelines. These technologies fall into three categories; physical, chemical and biological methods. Biological technologies available for cleanup of petroleum hydrocarbon polluted aquatic environment include the use of straw or plant material as oil absorbent, the addition of bio surfactants, and the addition of nutrients and bio-materials to encourage biodegradation of the

hydrocarbons (Jain *et al*., 2011; Okoh and Trejo-Hernandez, 2006). Physical and chemical/biochemical means used to encourage microbial degradation of hydrocarbons in open-waters include immobilization of hydrocarbon-absorbing materials such as alginate (Li *et al*., 1995), use of organisms encapsulated in wax (Rasnick, 1998), and use of microcapsule system and polyurethane foam (Oh, *et al*., 2000).

A physical procedure available for in situ bioremediation of hydrocarbon contaminated groundwater which involves the introduction of oxygen and nutrients is biosparging (Jain *et al*., 2011; Testa and Winegardner, 1991). In biosparging, air under pressure is injected below the water table. The injected air leads to increase in oxygen concentration in the water thereby stimulating the population increase of indigenous hydrocarbon degrading microorganisms. This leads to enhanced biodegradation of the hydrocarbon contaminants thereby leading to bioremediation of the groundwater environment. Oxygen is among the factors that limit activities of indigenous microflora (Devine, 1992).

Aeration is currently used in Brooklyn, NY, USA to remediate polluted urban waterways (Dueker and O'Mullan, 2014). Aeration increases the oxygen levels in the water column thereby leading to increased pollutant removal. Injection of air into subsurface near a BTEX-plume has been shown to cause the change of BTEX removal mechanisms from anaerobic biodegradation to aerobic biodegradation (Chen *et al*., 2010). Though biodegradation of petroleum hydrocarbons can occur in anaerobic conditions, the rate of biodegradation is slow compared to that occurring in aerobic conditions (Efeovbokhan *et al*., 2014). For enhanced remediation of crude oil polluted surface waters and groundwater a form of aeration is therefore important.
The aim of this research work is to remediate petroleum hydrocarbon polluted water through the application of aeration and bioaugmentation. The outcome of the study will assist bioremediation experts in making decision between the use of aeration alone and aeration plus bioaugmentation in the remediation of crude oil polluted water.

Materials and Methods

Experimental Setup

River water collected from the Eagle island river located behind Rivers State University, Port Harcourt, was used in the experimental setup. The river water was analyzed for total heterotrophic bacterial population, hydrocarbon utilizing bacterial population, phosphate and nitrate concentration, salinity, and turbidity.
The experimental setup was made up of three Erlenmeyer flasks of 2 L capacity. Each flask was filled with 1 L of the river water, and artificially contaminated with 10 ml crude oil. Air was bubbled into one of the flask through the use of an aquarium pump (Sea Star, HX-106A), the setup was labeled A; about 1 ml of a broth culture of *Pseudomonas* sp. was added to the content of the second flask, and air also bubbled into it. This setup was labeled A+M. The third flask was left as a control, and labeled Ctrl. The experimental setup was maintained for a duration of 28 days, and monitored at weekly intervals.
The *Pseudomonas* species used in the research was obtained from the stock cultures of Peekate P. L. which was isolated in a previous research of Peekate and Abu (2017).

Monitoring of Experimental Setup

Samples were collected from the flasks at weekly intervals. The samples were collected with the aid of sterile 10 ml pipettes. The samples were analyzed for Total heterotrophic bacterial population (THB), Hydrocarbon utilizing bacterial population (HUB), phosphate and nitrate concentration, and total hydrocarbon concentration (THC). THC was determined for the

samples, at the beginning and end of the experimental period, through spectrophotometric analysis. The THB were determined using Nutrient agar medium and the spread plate count method, while the HUB were determined using the mineral salt agar medium of Mills *et al.* (1978). The medium was compounded as shown in Table 1, sterilized in an Autoclave at 121 °C for 15 minutes, and allowed to cool to about 50 °C before addition of the antifungal agent Fluconazole. Crude oil was supplied into inoculated mineral salt agar plates using the vapour phase transfer method (Ebuehi *et al*., 2005), and the plates were incubated at ambient temperature (29 – 31 °C) for 5 – 7 days. Inoculated Nutrient agar plates for THB were incubated at 37 °C for 24 hours.

Spectrophotometric Analysis of the Samples for Quantification of THC

About 10 ml of the river water samples were placed, separately, in a 250 ml capacity separating funnel, followed by the addition of 20 ml Xylene. The mixture was agitated for about 5 minutes, and then allowed to stand for separation of the aqueous phase from the solvent phase. The aqueous phase was removed, while the solvent phase was collected and subjected to absorbance measurement using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China) set at 420 nm. The total hydrocarbon concentrations (THC) in the samples were then extrapolated from the absorbance reading of the solvent phase, with the aid of the equation of the straight line of the calibration graph previously obtained.

Table 1: Composition of the mineral salts medium used in the enumeration of hydrocarbon utilizing bacteria

Salts	**g.L^{-1}**
$MgSO_4.7H_2O$	0.42
KH_2PO_4	0.83
NaCl	10.0
KCl	0.29
Na_2HPO_4	1.25
$NaNO_3$	0.42
Agar	20.0

(**Source:** Mills *et al.*, 1978)

Isolation and Identification of Bacteria from the Experimental Setup

At the end of the study, selected bacterial colonies on enumerated nutrient agar plates for THB determination were isolated unto fresh Nutrient agar plates to obtain pure cultures. The bacteria isolates were selected based on difference in colonial morphology. Stock cultures of the isolates were prepared using Nutrient agar slants, and the isolates were subjected to Gram staining and microscopic examination. The following physicochemical/biochemical tests were carried out on the isolates: catalase, oxidase, motility, citrate utilization, indole production, Methyl Red-Vogues Proskauer (MRVP), blood haemolysis, casein hydrolysis, lecithinase production, and fermentation tests using glucose, lactose, maltose, sucrose, mannitol, xylose, starch, and glycerol.

Results

Bacterial Load and Physicochemical Properties of the River Water

The total heterotrophic bacterial population of the river water used in the experimental setup was 6.45×10^5 cfu/ml, hydrocarbon utilizing bacterial population was 1.37×10^3 cfu/ml,

phosphate concentration was 0.4 mg/L, nitrate concentration was 0.8 mg/L, salinity 13, 189.5 ppm, and turbidity was 9.0 NTU. From the data it can be seen that the population of the hydrocarbon utilizing bacteria were relatively low.

Bacterial population of the experimental setup

The total heterotrophic bacterial population (THB) and the hydrocarbon utilizing bacterial population (HUB) of the samples from the different flasks at weekly intervals are presented in Figure 1 and 2. In Figure 1, it can be seen that the THB population in setup A+M was higher in the course of the experimental period. However, HUB population of all the units in the experimental setup were almost in the same range as can be seen in Figure 2.

Changes in values of the monitored physicochemical parameters of the experimental setup

The phosphate and nitrate concentration, and the total hydrocarbon concentration (THC) at the beginning and end of the experimental period are presented in Figure 3 – 5. From the figures it can be seen that there is a general decrease in the physicochemical parameters monitored.

Identity of the bacteria isolates from the experimental setup

Bacteria isolated at the end of the study from the experimental setup were coded as follows: A1, A2, A3, A4, A5, A6, C1, C2, C3, C4, C5, C6, PS1, PS2, PS3, PS4, PS5, and PS6. Their microscopic morphology and reaction pattern to the biochemical/physicochemical tests used is presented in Table 2a and 2b. Based on comparison with literature (Madigan *et al*., 2000; Prescott *et al*., 1999; Stanier *et al*., 1977), their identities are suspected as follows: A1 & C5 – *Vibrio* sp.; A2, PS1, & C4 – *Bacillus* sp.; A3, C1, PS2, & PS5 – *Pseudomonas* sp.; A4, C6, & PS3 – *Neisseria* sp.; A5, C2, & PS4 – *Micrococcus* sp.; A6, C3, & PS6 – *Staphylococcus* sp.

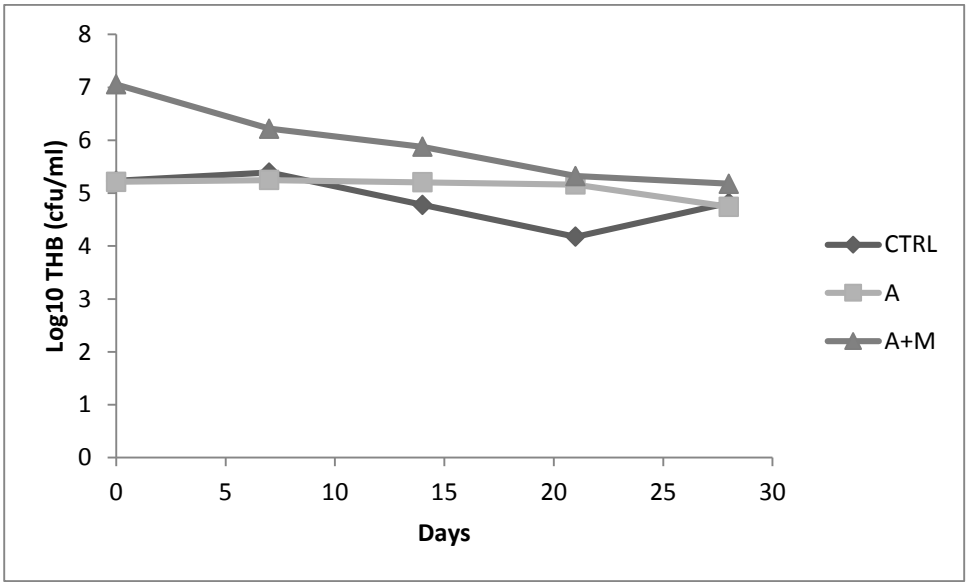

Figure 1: Total heterotrophic bacterial population (THB) of the experimental setup.
Key: Ctrl – Control, A – aeration only, A+M – aeration plus addition of *Pseudomonas*

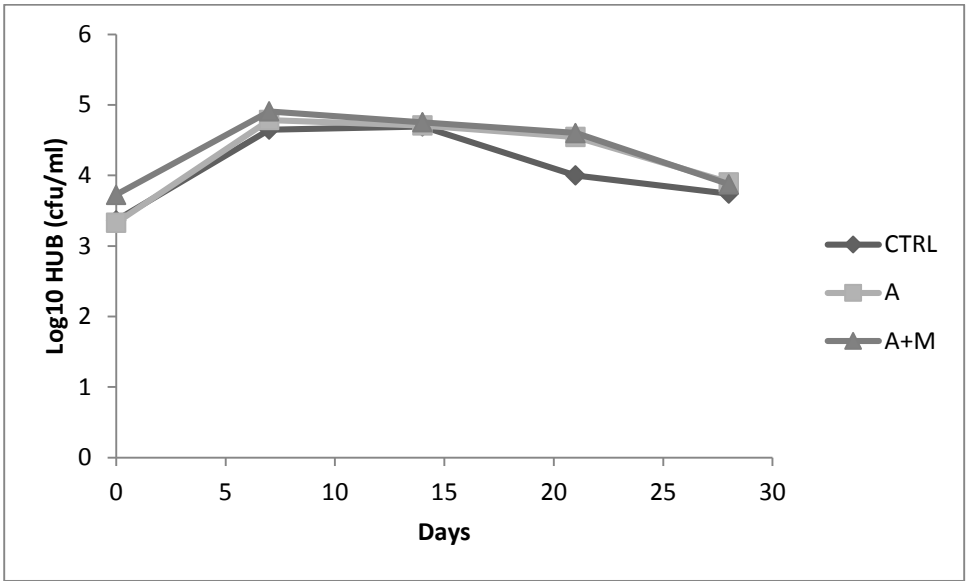

Figure 2: Hydrocarbon utilizing bacterial population (HUB) of the experimental setup.

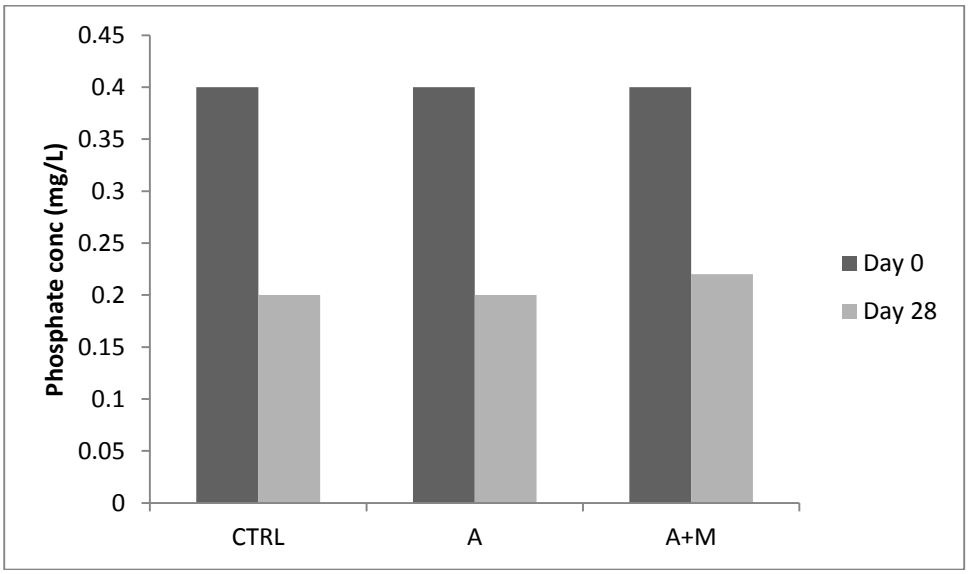

Figure 3: Phosphate concentration of the experimental setup at the beginning and end of the experimental period.

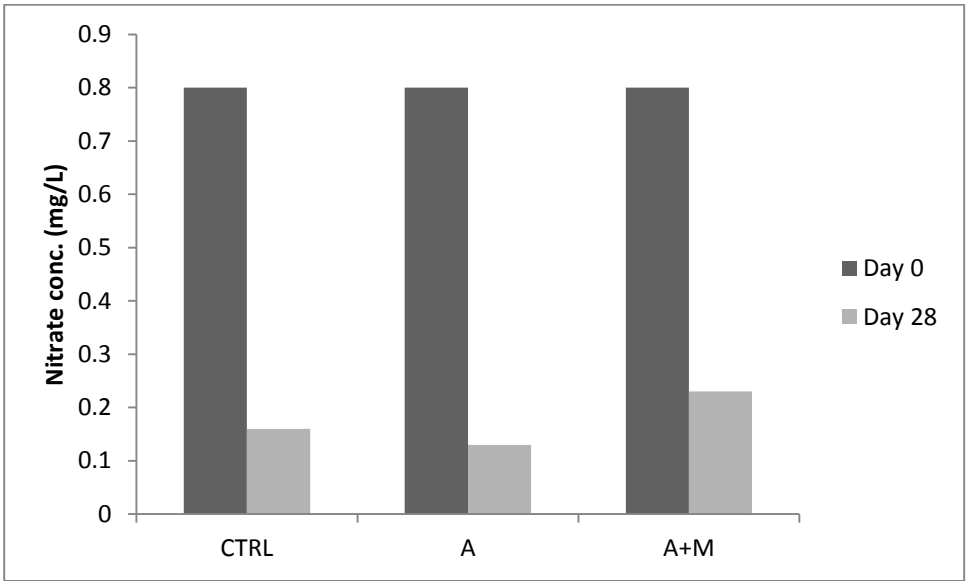

Figure 4: Nitrate concentration of the experimental setup at the beginning and end of the experimental period.

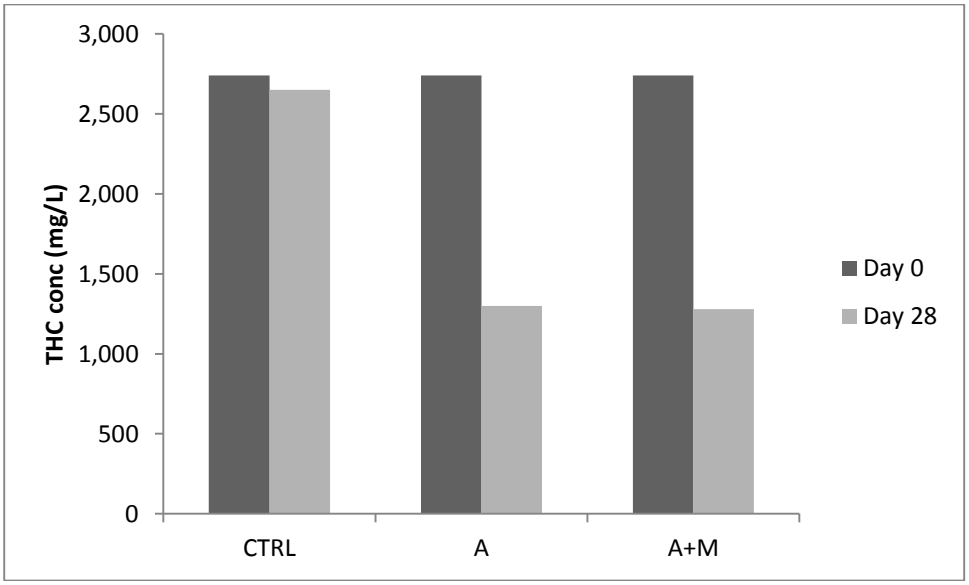

Figure 5: Total hydrocarbon concentration (THC) of the experimental setup at the beginning and end of the experimental period.

Table 2a: Identification results of the bacterial isolates from the experimental setups

	A1	A2	A3	A4	A5	A6	C1	C2	C3
Gm/M	- r	+ r	- r	- c	+ c	+ c	- r	+ c	+ c
Ctl	+	+	+	+	+	+	+	+	+
Oxd	-	-	+	+	+	-	+	+	-
Mtl	+	+	+	-	-	-	+	-	-
CtU	-	+	+	-	-	+	+	-	+
Ind	+	-	-	-	-	-	-	-	-
VP	-	+	-	-	+	+	-	+	+
MR	+	+	-	+	-	-	-	-	-
HBA	β-H	β-H	β-H	γH	γH	βH	β-H	γH	βH
CsH	-	+	+	-	-	-	+	-	-

LcP	-	+	-	-	-	-	-	-	-
GluF	A	A	A	A	0	A	A	0	A
LtF	0	0	0	0	0	A	0	0	A
XsF	0	0	A	A	0	0	A	0	0
MalF	A	A	0	A	0	A	0	0	A
MntF	A	0	A	0	A	A	A	A	A
SucF	0	A	0	A	0	A	0	0	A
StaF	0	A	0	A	0	0	0	0	0
GlyF	0	0	A	0	0	A	A	0	A

Gm/M – Gram stain/Morphology, Ctl – catalase, Oxd – oxidase, Mtl – motility, CtU – citrate utilization, Ind – indole, VP – Vogues Proskauer, MR – Methyl red, HBA – haemolysis on blood agar, β-H – beta haemolysis, γH – gamma haemolysis, CsH – Casein hydrolysis, LcP - lecithinase production, GluF – Glucose fermentation, LtF – Lactose fermentation, XsF – Xylose fermentation, MalF – Maltose fermentation, MntF – Mannitol fermentation, SucF – Sucrose fermentation, StaF – Starch fermentation, GlyF – Glycerol fermentation, A – acid produced, 0 – no change.

Table 2b: Identification results of the bacterial isolates from the experimental setups

	C4	**C5**	**C6**	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**
Gm/M	+ r	- r	- c	+ r	- r	- c	+ c	- r	+ c
Ctl	+	+	+	+	+	+	+	+	+
Oxd	-	-	+	-	+	+	+	+	-
Mtl	+	+	-	+	+	-	-	+	-
CtU	+	-	-	+	+	-	-	+	+
Ind	-	+	-	-	-	-	-	-	-
VP	+	-	-	+	-	-	+	-	+
MR	+	+	+	+	-	+	-	-	-
HBA	β-H	β-H	γH	β-H	β-H	γH	γH	β-H	βH
CsH	+	-	-	+	+	-	-	+	-
LcP	+	-	-	+	-	-	-	+	-
GluF	A	A	A	A	A	A	0	A	A
LtF	0	0	0	0	0	0	0	0	A
XsF	0	0	A	0	A	A	0	0	0
MalF	A	A	A	A	0	A	0	0	A
MntF	0	A	0	0	A	0	A	0	A
SucF	A	0	A	A	0	A	0	0	A
StaF	A	0	A	A	0	A	0	0	0
GlyF	0	0	0	0	A	0	0	A	A

Discussion

Petroleum hydrocarbon polluted aquatic environment poses a threat to flora, fauna and humans, thus the need to remediate such environment. The use of aeration alone and aeration plus bioaugmentation is investigated in this study as a means to remediate petroleum hydrocarbon polluted water. The data obtained from the study showed that about 52.6 % reduction in total hydrocarbon concentration (THC) was obtained using only aeration, while about 53.3 % reduction in THC was obtained using aeration plus bioaugmentation. There was thus no much difference between the two methods. This implies that of the two methods investigated, it would be economical to use the aeration method alone in the remediation of crude oil polluted

water. Reduction of THC of about 93.6 % has been achieved in a study where aeration and addition of nutrients and mixed microbial culture was used in the bioremediation of crude oil polluted water (Amenaghawon *et al*., 2014). The high reduction in the study compared to that obtained in this study can be attributed to only the addition of nutrients. Addition of microorganisms to water systems for bioremediation has been reviewed to be unnecessary (Chapelle, 1999). This is confirmed in this study; for the addition of *Pseudomonas* sp. made no much difference. Also the relatively high reduction in THC in the study of Amenaghawon *et al*., (2014) could be attributed to the duration of their experiment; it took 56 days to achieve such reduction. In another study on bioremediation of polluted water it took 35 days to achieve a reduction of 98.8 % (Efeovbokhan *et al*., 2014). In this study, the duration of the remediation experiment was 28 days; lower compared to the others.

The close variation in the population of the hydrocarbon utilizing bacteria population in the aeration setup and the aeration plus bioaugmentation setup correlates with the close reduction in THC in both setups. This can be observed by comparing Figure 2 and 5. The bacteria isolated at the end of the study which include *Vibrio* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., and *Staphylococcus* sp. have been shown to degrade hydrocarbons (Leahy and Colwell, 1990; Chikere *et al*., 2009). These bacteria were isolated from all the setup, except *Vibrio* sp. which was not isolated from the aeration plus bioaugmentation setup. It probably was missed during sub-culturing from enumerated plates. The presence of all the isolated bacteria which are hydrocarbon degraders in the different setup confirms the reduction in hydrocarbon concentration, and also further confirms that there was no need in adding microorganisms as in the case of aeration plus bioaugmentation.
Physicochemical evidence for bioremediation of the petroleum hydrocarbon polluted water in this study is provided for by the decrease in nitrate and phosphate concentration (Figure 3 and 4). Depletion of nitrate has been cited as an evidence for the occurrence of intrinsic bioremediation within hydrocarbon plumes (Chen et al., 2010).

Conclusion

With the various technologies available for remediation of crude oil polluted aquatic environment, and their inherent cost, it becomes imperative to come up with a cost effective solution. The use of aeration alone in the remediation of polluted aquatic environment entails simple supply of air with the aid of air pressure pumps which can be operated mechanically or electrically. Other forms of remediation of polluted aquatic environments involves the use of surfactants/biosurfactants, nutrient application, and bio-materials. Factoring in the expenses associated with surfactant/biosurfactant production, and pathogens that may be associated with biomaterials, it becomes a thing of simplicity to apply aeration.
In this research work it has been shown that there is no difference between the use of aeration and aeration plus bioaugmentation in the remediation of petroleum hydrocarbon polluted water. The use of aeration alone is recommended over the use of aeration and bioaugmentation for remediation of crude oil polluted aquatic environment. Future research is suggested in the area of nutrient addition with aeration for bioremediation of crude oil polluted aquatic environment.

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