

# BIODEGRADATION AND KINETIC STUDIES OF GROUNDWATER CONTAMINATED WITH DOMESTIC PURPOSE KEROSENE USING MICROORGANISMS FROM AGRICULTURAL WASTE

BY

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## ABSTRACT

This study investigated the microbial degradation and kinetic study of groundwater contaminated with domestic purpose kerosene (DPK) by mixed-culture of microorganisms at ambient temperature (28-32°C). The results obtained showed that the microorganisms; total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungi (TF) had positive response in utilizing total petroleum hydrocarbon (TPH) as domestic purpose kerosene. The order of hydrocarbon utilization was: THUB > TF > THB with mean growth rate constants of  $3.96 \times 10^{-3} \text{ hour}^{-1}$ ,  $1.98 \times 10^{-3} \text{ hour}^{-1}$  and  $1.48 \times 10^{-3} \text{ hour}^{-1}$  in that order. A total of ten bacterial isolates were recovered from the total viable count (TVC) plate. Six of these were hydrocarbon degraders. Four (4) fungal species were recovered. The control microcosm showed a reduction in TPH by 2.5% probably due to activities of microbes initially present in the groundwater sample or evaporation, as experiments were carried out in open - system to mimic natural environmental conditions. The degradation followed pseudo-first order kinetics with rate constant of  $1 \times 10^{-2} \text{ mg}^{-1} \text{ Lhr}^{-1}$  at room temperature. In conclusion, the native microorganisms from the agricultural waste of poultry, piggery and cow had the capability of degrading petroleum hydrocarbons in areas where oil spillages occur.

**Keywords:** agricultural waste, microorganisms, utilization, biodegradation, kinetics.

## INTRODUCTION

The United Nations, Educational, Scientific and Cultural Organization (UNESCO) (1994) reported that about 1.2 billion people globally lack safe drinking water and about 50% of the populations in developing countries do not have access to safe and sustainable sources of water supply. Groundwater has been reported to account for about 88% of safe drinking water where the population is widely distributed and treatment plants and transport facilities for surface water are non-existent (Kumar, 2004). Unfortunately, groundwater has been known to be contaminated through various ways these include seepage from effluent waters, fertilizer from agricultural

activities, mining activities, vehicle maintenance, sewage disposal, domestic waste and oil spills (Shyamala *et al.*, 2008). According to Obire and Putheti, (2009), 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, refining and transportation which in turn has resulted in gross pollution of the environment. Accidents in which spills cause serious damages to the environment are widely reported (Gbadagesin, 1997; Ekekwe, 2010). Oil spills from such accidents may quickly spread over many square miles of water surface and groundwater. Dubbed as the bloodline of modern civilization, petroleum-hydrocarbon composition varies

greatly in its complex mixture of hydrocarbons and other organic and inorganic compounds, which contribute to the diversity in its physical properties (Van Hamme *et al.*, 2003).

The dependence of humans on oil and its derivatives around the world leads to inevitable spillages. This has called for more studies into oil pollution problems (Jain *et al.*, 2011). In recent years a lot of bioremediation work on soil and water using microorganisms have been reported (Van Hamme *et al.*; Piskonen and Itävaara, 2004; Venosa *et al.*, 1996; Medjor *et al.*, 2012). However, kinetic studies on the microbial biodegradation of groundwater contaminated with domestic purpose kerosene (DPK) using microorganisms from agricultural waste are limited or lacking. This work is therefore aimed at investigating the microbial biodegradation and kinetics of groundwater contaminated with domestic purpose kerosene (DPK) using microorganisms from agricultural waste.

## MATERIALS AND METHODS

### Materials

The petroleum contaminant used in the study was domestic purpose kerosene (DPK). It was obtained from Petroleum Products Marketers in Agbor, Delta State, Nigeria. All plastics and glassware utilized were pre-washed with detergent water solution, rinsed with tap water and soaked for 48 hours in 50% HNO<sub>3</sub>, then rinsed thoroughly with distilled water and air-dried in the laboratory.

### Sampling method

Groundwater sample was obtained in pre-sterilized 10 litre container from the tap of a borehole located in Delta State University, Abraka, Nigeria. In the early hours of the morning, the borehole tap was allowed to run for about 2-3 minutes to eliminate impurities and then snap-shot sample was taken at once (grab sampling),

(Adeomoroti, 1996). The groundwater sample was immediately taken to the Microbiology laboratory within the University for the Bioremediation Experiment.

### Bioremediation Experiment

Two sets of experiments were carried out at ambient temperature (28-32 °C) following similar procedures of oil application method that has been reported elsewhere (Venosa *et al.*, 1996). In the first experiment, 25g of the organic supplement that served as a source of microorganisms and nutrients was added to the prepared groundwater sample contaminated with domestic purpose kerosene in a plastic container. In the second experiment, a second plastic container containing prepared groundwater sample contaminated with domestic purpose kerosene was left without organic supplement and acted as a control. The experimental set up was allowed to stand for 42 days for possible bioremediation. Bioremediation indicators monitored included changes in: changes in total petroleum hydrocarbon (TPH), total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungi (TF). Isolation and enumeration of heterotrophic, hydrocarbon utilizing bacteria and fungi was carried out using the spread plate technique as described by ALPHA, (1998). Fungal isolates were identified using the method of Harrigan and McCane (1976). Characterizations of isolates (Gram staining reaction, Citrate utilization test, Motility test, Spore staining, Oxidase test, Catalase test, Methyl red/Voges-Proskauer (Mrvp), Indole production test, Oxidation and fermentation of sugars and Urease hydrolysis were carried out adopting methods described by Stewart and Beswick (1977) and Cruickshank *et al.*, (1975).

The growth rate during the exponential phase was expressed in terms

of the mean growth rate constant ( $k$ ) using the formula:  $n = (\log N_t - \log N_0)/0.301$  and  $k_1 = n/t$  where

$N_0$  = population number at lag phase;  $N_t$  = population number at log phase,  $n$  = the number of generations in time  $t$  and  $t$  = time to attain the log phase. The mean generation time ( $g$ ) is the reciprocal of the mean growth rate constant,  $g = 1/k_1$  (Prescott *et al.*, 2008).

The kinetics of bioremediation was also studied. Changes in total petroleum hydrocarbon (TPH) with time were monitored on weekly basis in the domestic purpose kerosene microcosm for 6 weeks. TPH analyses were carried out using T-60 UV/Visible spectrophotometer (2007 model). Biogenic factor (TPH contributions from the organic supplement) was neglected and therefore total hydrocarbon content (THC) was considered as total petroleum hydrocarbon (TPH).

## RESULTS AND DISCUSSION

A total of 15 bacterial isolates were recovered from the total viable count (TVC) plate, of these six were hydrocarbon degraders. The genera of the following indigenous bacteria were identified: *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Corynebacterium* spp., *Flavobacterium* spp., *Alcaligenes* spp. and *Cellulomonas* spp., *Chromobacterium* spp., *Serratia* spp., and *Proteus* spp. The fungal isolates obtained were *Candida* spp., *Aspergillus* spp., *Detorula* spp and *Tricoderma* spp.

The initial population of the total heterotrophic bacteria (THB) in the domestic purpose kerosene microcosm was  $57.00 \times 10^5$  CFU/mL. After 336 hours the population of the THB dropped to  $3.50 \times 10^5$  CFU/mL. This accounted for 93.86% decrease in population of the THB in the domestic purpose kerosene medium. The heterotrophic bacteria experienced an unbalanced growth being transferred from a rich-amendment into nutritionally poor

water-medium polluted with domestic purpose kerosene. During the exponential or log phase, a peak growth of  $6.10 \times 10^5$  CFU/mL after 672 hours after the initial devastation had occurred. This represented an exponential growth rate of 174.29%. The addition of organic amendment substantially accelerated biodegradation by providing the necessary support and stimulation for native microbes to quickly multiply and produce enzymes to speed up the metabolic process of biodegradation. To adapt to their new environment, enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume (Geo-Brooks *et al.*, 2010). The adjustment of carbon/nitrogen/phosphorus ratios by addition of nitrogen and phosphorus in the form of N-P-K fertilizer simulated the biodegradation of crude oil and individual hydrocarbon has been widely reported (Zhu *et al.*, 2001; Okpokwasili and Oton, 2006). Bioremediation process typically involves many different microbes acting in parallel or sequence to complete the degradation process. Similar trends of the lag, log and death phases were exhibited by the total hydrocarbon utilizing bacterial and fungal populations as observed by heterotrophic bacteria. The initial population of the total hydrocarbon utilizing bacteria in the kerosene microcosm was  $3.10 \times 10^4$  CFU/mL. After 168 hours the population of the THUB had dropped to  $0.94 \times 10^4$  CFU/mL. This accounted for 69.68% decrease in population of the THUB. The growth peak of the total hydrocarbon utilizing bacteria (THUB) was  $2.80 \times 10^4$  CFU/mL, accounted for 297.87% increase after 504 hours. The initial population of fungi in the groundwater sample polluted with domestic purpose kerosene was  $1.30 \times 10^4$  CFU/mL. There was no drop in the initial fungal population. An exponential increase in fungal population by 715% was observed at the third week (504 hours) before the death phase set in at the fifth

week (672 hours) through the seventh week (1008 hours). This is generally contrary to expectations as when microorganisms are introduced into fresh culture medium, usually no immediate increase in cell occurs (lag phase). 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 microorganism from the 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 504 hours grew better than the bacterial population. This is in conformity with the study done by Al-Daher *et al.*, (1998) where it was reported that fungi are more tolerant in acidic conditions. The stationary phase was absent in all cases because experiments were conducted in open systems. The growth rates for heterotrophic, hydrocarbon utilizing bacteria and fungi are represented in a growth profile in Figure 1.

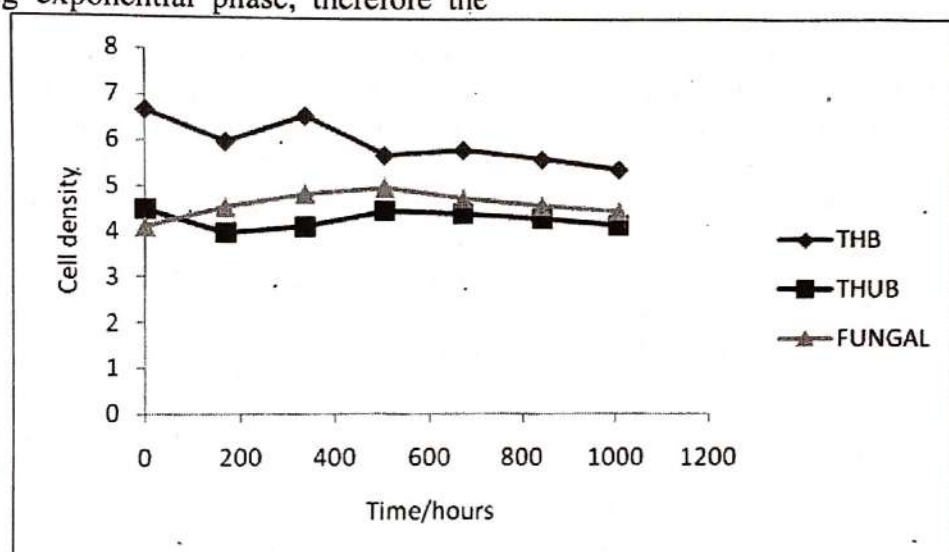


Figure 1: Microbial population (cell density) in a bioremediation experiment using organic amendments in water contaminated with domestic purpose kerosene. The mathematics of growth for the total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungal (TF) population is given in Table 1.

Table 1: Data for mathematics of growth for the total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB) and total fungal (TF) population in domestic purpose kerosene medium

Type	Population number at lag phase ( $N_0$ )/CFU/mL	Population number at log phase ( $N_t$ )/CFU/mL	n	$k_1$ ( $\text{hr}^{-1}$ )	g (hr/generation)
THB	$3.50 \times 10^5$	$6.10 \times 10^5$	1	$1.48 \times 10^{-3}$	676
THUB	$0.94 \times 10^4$	$2.80 \times 10^4$	2	$3.96 \times 10^{-3}$	253
TF	$1.30 \times 10^4$	$9.30 \times 10^4$	1	$1.98 \times 10^{-3}$	505

The order of hydrocarbon utilization was: THUB >TF>THB with mean growth rate constants of  $3.96 \times 10^{-3} \text{ hr}^{-1}$ ,  $1.98 \times 10^{-3} \text{ hr}^{-1}$  and  $1.48 \times 10^{-3} \text{ hr}^{-1}$  respectively. The THUB population had the shortest mean generation time of 253 hours followed by the TF population with a mean generation time of 505 hours and the THB population had the longest mean generation time of 676 hours. Generation times vary markedly with the species of microorganism and the environmental conditions as shown in this work and corroborated by the findings of earlier investigators (Prescott *et al.*, 2008). Analysis of variance (ANOVA) of the results of the populations of total hydrocarbon utilizing bacteria (THUB), total heterotrophic bacteria (THB) and fungal used in the study showed significant differences at  $p < 0.05$ . This statistical finding supported the fact that the total hydrocarbon utilizing bacteria, total heterotrophic bacteria and fungi populations had different utilization capability for the domestic purpose kerosene as a source of carbon for food as also indicative by the mathematics of growth in Table 1. The increase in the 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 Amadi, (1992) high population sizes suggest increase in hydrocarbon assimilation. According to Odu, (1981) and Agbonlahor, (2004) the relative significance of bacterial to fungal population in hydrocarbon degradation has been an issue of controversy. The study done by Al-Daher *et al.*, (1998) also reported that fungi are more tolerant in acidic conditions. 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 was also very evident in this study as it was difficult to clearly ascertain whether bacteria or fungi played the predominant role during the bioremediation process considering the fact that while the total hydrocarbon utilizing bacteria thrived better than the fungal and total heterotrophic bacteria population; the fungal population on the other hand had better utilization capacity than the total heterotrophic bacteria population. Many microorganisms have been found to have the ability to utilize hydrocarbons as sole source of energy and such microorganisms are widely distributed in nature (Atlas, 1981).

Temperature is a measure of the degree of hotness or coldness of a substance. The degree of hotness of a sample affects the aquatic organisms living in it. The temperature readings of the experiments were in the mesophilic range of  $28-32^{\circ}\text{C}$ , this is the ambient temperature in Nigeria (Eziuzor and Okpokwasili, 2009). Low temperature has been reported to affect microbial growth and propagation, and under normal circumstances, rates of degradation decrease accordingly as a result of primarily decrease in the rates of enzymatic activity (Gibb *et al.*, 2001). The optimum temperature is typically in the range of  $30$  to  $40^{\circ}\text{C}$ . At temperature above this norm, enzymatic activities are inhibited as proteins denatured (Leahy and Colwell, 1990). Microbial growth and activities are also readily affected by pH, temperature, and moisture. Although microorganisms have also been isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions (Vadalli, 2001). The mean pH of the uncontaminated groundwater sample was 7.3. During the first week, the pH value showed sharp depression at 5.30. The change indicated a drift from the slightly

alkaline to slightly acidic condition. The result is represented in Figure 2.

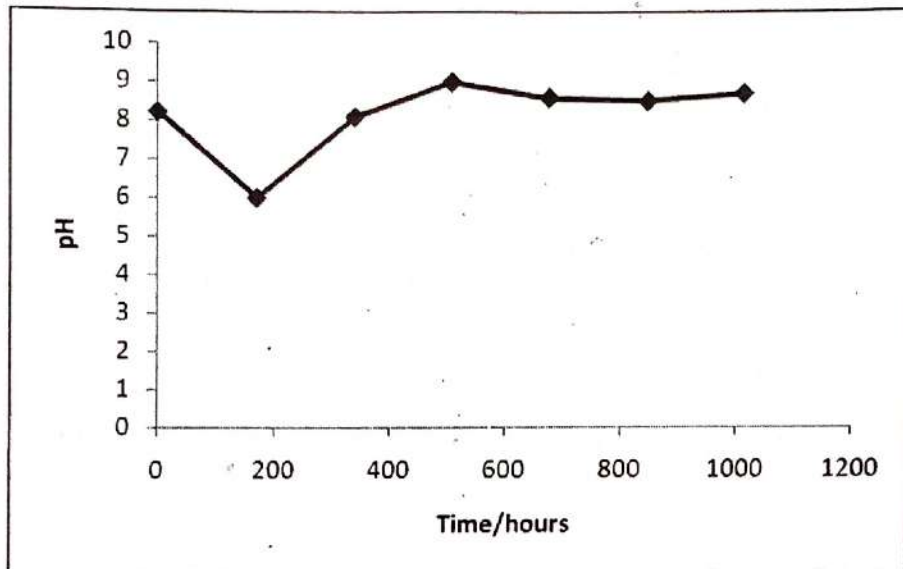


Figure 2: pH profile in a bioremediation experiment using organic amendments in water contaminated with domestic purpose kerosene

This acidic condition contributed immensely to the high death rate of bacteria as a result of simultaneous production and accumulation of acidic metabolic products particularly during the first week of the experiment and accounted for the drop in the pH. The result was consistent with the findings of Okpokwasili and Nnorom, (1986) and Agbonlahor, (2004). The subsequent rise in pH provided a slightly alkaline condition (8.70) that enhanced microbial activity. pH has been reported to be critical to the effectiveness of biodegradation. The pH variation in the alkaline range is likely due to the high amount of organic supplement used to biostimulate the microbes for effective microbial

degradation. The presence of nitrogen in the form of ammonia or ammonium ions in the organic supplement may have also strongly influenced the pH towards alkalinity. The pH of the domestic purpose kerosene contaminated groundwater sample after bioremediation process at the sixth week was found to be 8.7. The pH value was significantly higher compared to that of the uncontaminated groundwater sample (pH = 7.3).

Kinetic of bioremediation method was investigated for its order of reaction. Typical pseudo-first-order rate plot for the remediation process was tested by plotting  $\ln[B]_0 - \ln[B]_t$  against time using the experimental kinetic data and the outcome represented in Figure 3.

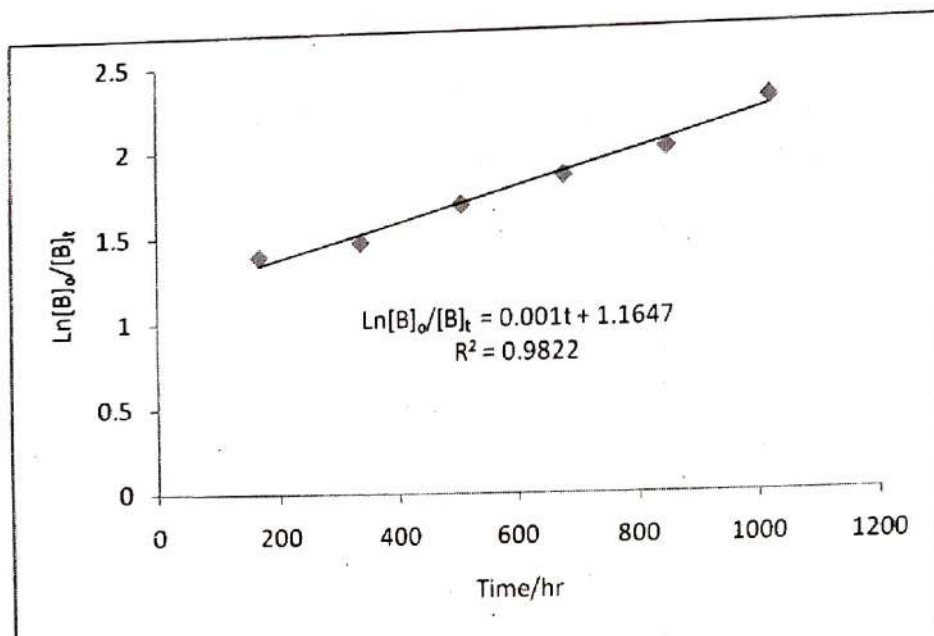


Figure 3: Pseudo- first order plot for TPH as domestic purpose kerosene in a bioremediation experiment using organic amendments in water contaminated with domestic purpose kerosene

The result of the plot graphically showed good linearity as regression coefficient was found to be  $> 0.9$  and the kinetic data adequately fitted into the pseudo- first order kinetic model and satisfied the conditions for pseudo-first order reaction. The slope of the graph gave the rate constant,  $k_2$ . The pseudo- first order rate constant was  $1.0 \times 10^{-2} \text{ mg}^{-1} \text{ Lhr}^{-1}$ . The control microcosm showed a reduction in TPH by 2.5% probably due to activities of microbes initially present in the groundwater sample or evaporation, as experiments were carried out in open - system to mimic natural environmental conditions.

### CONCLUSIONS

In conclusion, the study established that indigenous microorganisms readily available in mixed waste of poultry, piggery and cow are effective in utilizing and degrading total petroleum hydrocarbon as domestic purpose kerosene. The groundwater contaminated with domestic purpose kerosene exhibited pseudo-first order kinetics with very high remediation efficiency. The kinetics also provided valuable information to accurately predict time and efficiency of bioremediation using microorganisms from agricultural

waste for groundwater contaminated with domestic purpose kerosene.

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