International Journal of Environmental Bioremediation & Biodegradation, 2015, Vol. 3, No. 2, 48-53
Available online at http://pubs.sciepub.com/ijebb/3/2/2

DOI:10.12691/ijebb-3-2-2

Enhanced Aerobic Biodegradation of Naphthalene in Soil: Kinetic Modelling and Half-Life Study

S. E. Agarry*, K. M. Oghenejoboh

Biochemical and Bioenvironmental Engineering Laboratory, Department of Chemical Engineering, Delta State University, Oleh Campus, Nigeria
*Corresponding author: sam_agarry@yahoo.com

Received January 23, 2015; Revised May 10, 2015; Accepted June 07, 2015

Abstract To demonstrate the potential use of bioremediation in polycyclic aromatic hydrocarbons contaminated soil using naphthalene as a model pollutant, a laboratory study with the objectives of investigating, evaluating and comparing the methods of natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation was performed. The study dealt with naphthalene biodegradation in soil using inorganic NPK fertilizer and mixed culture of *Alcaligenes*, *Aeromonas*, *Micrococcus*, and *Serratia* as source of biostimulation and bioaugmentation, respectively. Each treatment strategy contained 4% (*w/w*) naphthalene in soil as a sole source of carbon and energy. After 4 weeks of remediation, the results revealed that natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation exhibited 44%, 69.5%, 77.5%, and 85% naphthalene degradation, respectively. Also, the total hydrocarbon-degrading bacteria (THDB) count in all the treatments increased throughout the remediation period. The highest bacterial growth was observed for combined biostimulation and bioaugmentation treatment strategy. A first-order kinetic model was fitted to the biodegradation data to evaluate the biodegradation rate and the corresponding half-life time was estimated. The model revealed that naphthalene contaminated-soil microcosms under combined biostimulation and bioaugmentation treatment strategy had higher biodegradation rate constants, k as well as lower half-life times, $t_{1/2}$ than other remediation systems. Therefore, the kinetic parameter values showed that the degree of effectiveness of these bioremediation strategies in the cleanup of naphthalene contaminated soil is in the following order: natural attenuation < biostimulation < bioaugmentation < combined biostimulation and bioaugmentation. Thus, the present work will contribute to the development of strategies for *in situ* treatment of polycyclic aromatic hydrocarbons contaminated soils.

Keywords: *bioremediation, biostimulation, bioaugmentation, first-order kinetics, naphthalene*

Cite This Article: S. E. Agarry, and K. M. Oghenejoboh, "Enhanced Aerobic Biodegradation of Naphthalene in Soil: Kinetic Modelling and Half-Life Study." *International Journal of Environmental Bioremediation & Biodegradation*, vol. 3, no. 2 (2015): 48-53. doi: 10.12691/ijebb-3-2-2.

1. Introduction

Soil is a valuable resource as it regulates biogeochemical cycles, filters and remediates pollutants and enables food production [1]. The presence of polycyclic aromatic hydrocarbons (PAHs, fused-ring compounds) in soil has considerable toxicological concern because of their high toxicity, mutagenic and carcinogenic properties [1,2]. Due to their high mobility and long persistence in the environment, these compounds are included in the list of priority toxic pollutants of the European Environmental Agency [3] and the US environmental protection agency [4]. PAHs are produced during fossil fuel combustion, waste incineration or as by-products of industrial processes including coal gasification, production of aluminum/iron/steel, creosote, pharmaceutical wastes, petroleum refining, and component of wood preservatives, smoke houses and wood stoves [5,6]. Systematic exposure to naphthalene (example of PAHs) and its derivatives has been shown to cause several diseases and disturbances to human metabolism [7,8]. Biological method of treatment has turned out to be a favorable alternative for naphthalene degradation and several reports are available on the removal of naphthalene by different microorganisms such as *Pseudomonas* spp, *Bacillus spp.*, *Vibrio* spp, *Mycobacterium* spp, *Marinobacter spp*, and *Sphingomonas spp.*, *Micrococcus spp* [9,10,11,12].

The aerobic and anaerobic biodegradation of PAHs have been studied [13,14,15,16] and were found to generally degrade slowly in the environment particularly in marine systems [17,18]. The low degradation rates of PAHs may probably be due to their stable structure, nutrient deficits in the environment and adsorption unto particulate matter [19,20,21]. Solid-phase ex situ soil bioremediation systems includes land farming, biopiling and composting. In most cases, the treatment of petroleum hydrocarbon contaminated environments has involved biostimulation—the addition of nutrients to stimulate the spontaneous enrichment of the indigenous hydrocarbon oxidizing microbial population [22].

Studies have revealed that biodegradation of PAHs can be enhanced with the use of biostimulating agents such as surfactants and inorganic or organic nutrients [13,14,15,16,23,24]. However, there has been considerable debate over the efficacy of bioaugmentation (the addition of dried or liquid cultures of either indigenous or exogenous microorganisms) [25] to expedite remediation process. This is because in some cases indigenous bacteria have been shown to out-compete artificially introduced strains in several bioremediation investigations [26].

Therefore, the objectives of this study were to examine, evaluate, and compare the methods of natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation in the bioremediation of soil contaminated with naphthalene. The kinetics of naphthalene biodegradation process was modeled as well as estimation of the biodegradation half-life time was carried out.

2. Materials and Methods

2.1. Soil Sample Collection

Soil samples were collected from an unimpacted zone of Ladoke Akintola University of Technology Agricultural Farm. About 2kg of bulk surface and subsurface soil was collected from different areas on the sampling sites. The bulked composite soil was preserved by putting them in sterile polyethylene bag, properly sealed and kept in the refrigerator prior to further use.

2.2. Soil Characterisation

The soil sample was characterized for total organic carbon (TOC), total nitrogen (N), total phosphorus, moisture content, pH, and total hydrocarbon degrading bacteria (THDB) according to standard methods. Soil pH was determined according to the modified method of McLean [27]; total organic carbon was determined by the modified wet combustion method [28] and total nitrogen was determined by the semi-micro-Kjeldahl method [29]. Available phosphorus was determined by Brays No. 1 method [30] and moisture content was determined by the dry weight method. The total hydrocarbon degrading bacteria (THDB) populations were determined by the vapor phase transfer method [31]. The physic-chemical properties of the soil are given in Table 1. The soil was characterised by low level of total nitrogen suggesting low nutrient level.

Table 1. Physico-chemical Characteristics of the Unimpacted Soil

Parameters	Value
Total nitrogen (%)	0.06 ± 0.01
Total carbon (%)	0.45 ± 0.03
Total organic matter (%)	0.78 ± 0.02
pH	7

2.3. Isolation, Characterization, and Identification of Bacteria

The bacterial strains used for bioaugmentation in this study were isolated from spent lubricant-contaminated soils collected from an Automobile Service Workshop in Ogbomoso, Nigeria. Soil samples were collected at subsurface level at five different points, pooled together and stored in closed containers at 4°C prior to use. The spread plate technique [32] using nutrient agar (Oxoid) was employed for their isolation. The plates were incubated at 37°C for 18–24 h. Pure bacterial isolates were characterized and identified using various criteria, as described by Krieg et al. [33]. Pure isolates were transferred into nutrient agar slants stored at 4°C and served as the stock cultures for subsequent tests. Six predominant bacterial genera were identified, which include *Alcaligenes*, *Aeromonas*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Serratia*. Meanwhile, the microorganisms present in the LAUTECH agricultural soil were identified to be made up of mainly *Bacillus* and *Pseudomonas* species. However, the microbial species of *Alcaligenes*, *Aeromonas*, *Micrococcus*, and *Serratia* present in the auto-mechanic soil which are not found in the LAUTECH agricultural soil were used as test organisms for the bioaugmentation study.

2.4. Inoculum Preparation and Enrichment of Mixed Bacterial Culture

A loopful of each stock culture of *Alcaligenes*, *Aeromonas*, *Micrococcus*, and *Serratia* species were respectively inoculated into 250 ml Erlenmeyer flasks containing 100 ml of freshly prepared sterile nutrient broth medium (0.8%) made up of yeast extract 2.0 g/L, peptone 5.0 g/L, NaCl 5.0 g/L, and agar 15.0 g/L, incubated at 37°C for 48 h in an orbiter shaker (), and agitated at 120 rpm. Aliquots (50 ml) of each culture medium were centrifuged at 4900 ×*g* for 15 min. The cell pellets were washed three times in sterile normal saline and resuspended in 750 ml autoclaved normal saline (0.89% NaCl in distilled water). Ten milliliters of each cell suspension were mixed in a sterilized screw-capped test tube and this was the mixed bacterial culture. One milliliter of the cell suspension (mixed bacterial culture) was introduced into a 250 ml Erlenmeyer flask containing 100 ml of sterile nutrient broth supplemented with naphthalene (3% w/v) as the carbon and energy source was incubated for 48 h at 37°C in an orbiter shaker agitated at a speed of 120 rpm. After 48 h of growth, in the same manner, 1 ml of the culture was transferred aseptically to serially increasing concentration of naphthalene up to 10% (w/v). Cells were recovered by centrifugation and resuspended in normal saline to set an OD of 0.7 at 660 nm. This inoculum (10% w/v) was the one used for bioaugmentation to study the biodegradation of naphthalene in soil.

2.5. Experimental Design for the Bioremediation of Naphthalene-Spiked Soil

Four plastic bins used as bioreactors were prepared for each treatment, designated as bioattenuation (treatment A), biostimulation (treatment B), bioaugmentation (treatment C), and combined biostimulation and bioaugmentation (treatment D). Each bioreactor contained 1 kg of soil, contaminated and mixed well with 100 ml of 40 g of naphthalene (4% *w/w*) dissolved in methanol. The bioreactor under treatment B was amended with 2.56 g of NPK fertilizer (biostimulation), and the bioreactor under

treatment C was amended with 40 ml of inoculum (3.2×10^5 colony forming units [CFU] · g−1) (bioaugmentation), respectively. The bioreactor under treatment D was amended with both 40 ml of inoculum and 2.56 g of NPK fertilizer (combined biostimulation and bioaugmentation) [34]. The bioreactor or microcosm under inorganic NPK fertilizer treatment had a C:N:P ratio of 100:10:1 [35]. It was assumed that the aforementioned quantity of the inorganic fertilizer applied to the contaminated soil in the bioreactor was well worked to at least 15 cm depth in the reactor. Thus, the equivalent of 640 kg per hectare of inorganic fertilizer was applied to the reactor. These amounts supplied 128 kg per hectare of nitrogen for the 4-week. The bioreactor under treatment A was not amended with either NPK fertilizer and/or inoculum (natural bioattenuation). Soil in the bioreactor used as control experiment was sterilized three times by autoclaving at 121°C for 15 min. All the bioreactors with its contents were incubated at room temperature ($28^oC \pm 2^oC$) for four weeks. The water (moisture) content of soil in each bioreactor was adjusted every week by addition of sterile de-ionized water to a moisture holding capacity of 50%. In order to avoid anaerobic conditions, contents of the bioreactor were aerated by mixing every 3 days. Samples were taken every week and analyzed for residual naphthalene and total hydrocarbon–degrading bacteria, respectively. The experiments were carried out in triplicates.

2.6. Extraction and Residual Naphthalene Analysis

The samples were air-dried and naphthalene was recovered from the soil by soxhlet extraction using n-hexane as the solvent. The hexane extracts were each evaporated to 2ml in a temperature-controlled water bath. The extracts were analysed by gas chromatography for naphthalene. The gas chromatograph was carried out by the Hewlett Packard Model 5890 Series II equipped with flame ionisation detector. Nitrogen was used as the carrier gas at a pressure of 60 – 65psi. The initial column temperature was 68°C and held at this temperature for 2 min after which it was increased to 260°C at a heating rate of 12°C/min for 16 min. It was isothermally held at this temperature for 4 min and then increased to 320°C at a heating rate of 15°C/min for 4 min and maintained at this temperature for 8 min. The pressure of hydrogen and air are 35 and 45psi, respectively, with injector and detector temperatures at 300 and 320°C, respectively. Sample volume injected was 2µl [36]. Calibration curve for naphthalene was prepared using the standard solutions supplied by manufacturer of the equipment.

2.7. Kinetic Model Analysis

Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a petroleum hydrocarbon contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permit prediction of the level likely to be present at some future time. Petroleum hydrocarbon biodegradation rates are usually difficult to predict due to the complexity of the environment [37]. Nevertheless, biodegradation rate of organic compounds by microorganisms is often described by the equation as follows [38]:

$$q = \frac{q_m c}{k + c} \quad (1)$$

Where q is biodegradation rate, q_m is maximum specific biodegradation rate, c is the substrate concentration and k is half-saturation constant. If $c \ll k$; Eq. (1) can be reduced to:

$$q = \frac{q_m c}{k} \quad (2)$$

Eq. (2) is a typical first-order model. The use of first-order kinetics in the description of biodegradation rates in environmental fate models is common because mathematically the expression can be easily incorporated into the model [39]. Assuming $k_1 = (q_m / k)$ and integrating Eq. (2), the following relation of substrate concentration to time can be obtained as given in Eq. (3):

$$\ln c = a + k_1 t \quad (3)$$

2.7.1. Estimation of Biodegradation Half-Life Times

The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening [40], environmental fate modeling [41] and describing the transformation of pollutants [42,43]. Biodegradation half life times ($t_{1/2}$) are calculated by Eq. (4) [44,45]:

$$t_{1/2} = \frac{\ln 2}{k} \quad (4)$$

Where k is the biodegradation rate constant (day^{-1}). The half life model is based on the assumption that the biodegradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil [46].

2.8. Data Analysis

The data were subjected to one-way analysis of variance (ANOVA) at 5% probability. The data analysis was performed using statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Naphthalene Removal and Microbial Growth

Figure 1 shows the biodegradation profile of naphthalene as a function of remediation time for each bioremediation strategy, natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation.

From Figure 1, it is revealed that naphthalene biodegradation started very fast during the first week of remediation in all the treatments and slowly continued up to the fourth week (day 28). The concentration of naphthalene (4% *w/w*) was reduced to 2.25%, 1.22%,

9.0%, and 6.0% in 4 weeks of remediation and correspondingly 44%, 69.5%, 77.5%, and 85% naphthalene reduction was achieved under natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation treatments, respectively. This observation revealed that during the naphthalene biodegradation in soil, addition of inorganic NPK fertilizer and bacterial inoculums individually resulted in a more effective bioremediation response than the natural attenuation. Similar observations have been reported for kerosene [34,47] and spent engine oil [48,49]. Nevertheless, bioaugmentation strategy elicited a higher biodegradation of naphthalene than the biostimulation treatment. This is in agreement with the observation of Bento et al. [50] who reported that among the individual methods of natural attenuation, biostimulation, and bioaugmentation that were used for the remediation of a soil contaminated by diesel oil, bioaugmentation method elicited higher diesel oil degradation than others. In contrast, other workers have shown that biostimulation strategy enhanced the bioremediation of kerosene contaminated soil [34,47], crude oil contaminated soil [51], spent engine oil contaminated soil [49] and lubricating oil contaminated soil [45] than bioaugmentation. Generally, in this work, the combination of biostimulation and bioaugmentation treatment strategy (which have not been reported for naphthalene removal) showed relatively greater naphthalene reduction than other treatments during the whole period of remediation. A similar observation has been reported for kerosene [34]. An insignificant reduction in naphthalene concentration was observed in control setup. This reduction may be due to combined effects of abiotic degradation of naphthalene and volatilization losses. A similar observation has been reported for kerosene [34,47].

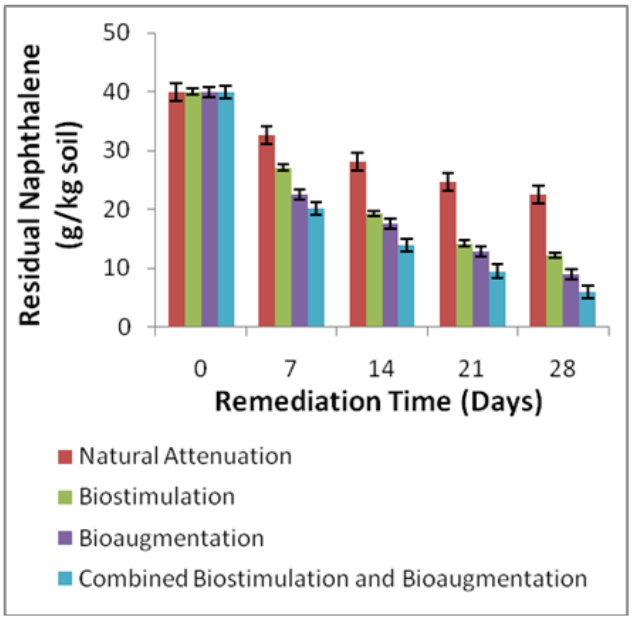

Figure 1. Time course for the biodegradation of naphthalene under (a) natural attenuation, (b) biostimulation, (c) bioaugmentation, and (d) combined biostimulation and bioaugmentation. Bars indicate the average of triplicate samples while the error bars show the standard deviation

Figure 2 shows the growth profile of the hydrocarbon degrading bacterial population in the soil. An initial hydrocarbon-degrading bacterial population of about 2.4×10^6, 2.6×10^6, 2.3×10^6, and 3.3×10^6 CFU/g of soil was observed respectively at the start of remediation for natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation treatments, as shown in Figure 2. This indicated that in the soil, the addition of preselected bacteria (bioaugmentation) and nutrients (biostimulation) resulted in the highest number of bacteria at the beginning of remediation compared to natural attenuation (or bioattenuation). The lower bacteria population in natural attenuation treatment may be due to the presence of low nutrients and the disturbance of nutrient ratio especially of NPK that is needed in the correct ratio for bacteria growth. As seen from Figure 2, a maximum bacterial population of 5.9×10^6, 9.9×10^6, 13×10^6, and 15.9×10^6 CFU/g of soil was respectively obtained under natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation treatment strategies at the end of the fourth week (day 28) of remediation.

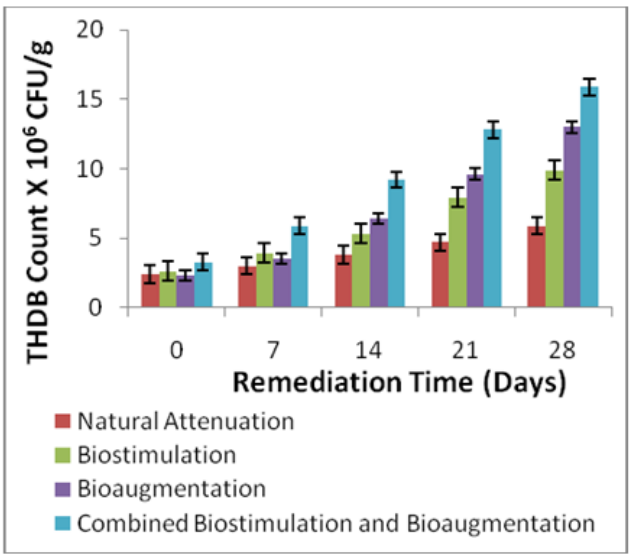

Figure 2. Time course for the growth of total hydrocarbon degrading bacteria (THDB) on naphthalene under (a) natural attenuation, (b) biostimulation, (c) bioaugmentation, and (d) combined biostimulation and bioaugmentation. Bars indicate the average of triplicate samples while the error bars show the standard deviation

3.2. Biodegradation Kinetics and Half-life

First-order kinetics model equation (Eq. 2) fitted to the biodegradation data (Figure 3) was used to determine the rate of naphthalene biodegradation in the soil treated through application of natural attenuation, bioaugmentation, biostimulation, and combined biostimulation and bioaugmentation, respectively.

The values of the rate constants obtained from fitting of the model are presented in Table 2.

The results in Table 2 as indicated by the high correlation determination (R^2) showed that the biodegradation of naphthalene fitted well to the first-order kinetic model. The half-life time of naphthalene biodegradation was calculated using Eq. (4). The biodegradation rate constants (k) and half-life times ($t_{1/2}$) for the different remediation treatments are presented in Table 2. It is to be noted that the higher is the biodegradation rate constants, the higher or faster is the rate of biodegradation and consequently the lower is the

half-life time. Table 2 shows that the biodegradation of naphthalene in soil under combined biostimulation and bioaugmentation treatment strategy had a higher k (0.064 day^{-1}) and lower $t_{1/2}$ (10.8 days) than that under bioaugmentation (k = 0.050 day^{-1} and $t_{1/2}$ = 13.9 days), biostimulation (k = 0.041 day^{-1} and $t_{1/2}$ = 16.9 days), and natural attenuation (k = 0.020 day^{-1} and $t_{1/2}$ = 34.7 days), respectively. Therefore, value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of soil contaminated with naphthalene is in the following order: bioattenuation < biostimulation < bioaugmentation < combined biostimulation and bioaugmentation.

Table 2. First-Order Kinetic Equation with Correlation Determination (R^2) Results of Naphthalene Biodegradation Under Different Bioremediation Methods

Bioremediation Methods	First-Order Kinetic Equation	k (day^{-1})	R^2	$t_{1/2}$ (days)
Biostimulation	$C = -0.041t + 3.616$	0.040	0.966	16.9
Bioaugmentation	$C = -0.050t + 3.590$	0.050	0.978	13.9
Combined Biostimulation and Bioaugmentation	$C = -0.064t + 3.580$	0.064	0.984	10.8
Natural Attenuation	$C = -0.020 + 3.652$	0.020	0.979	34.7

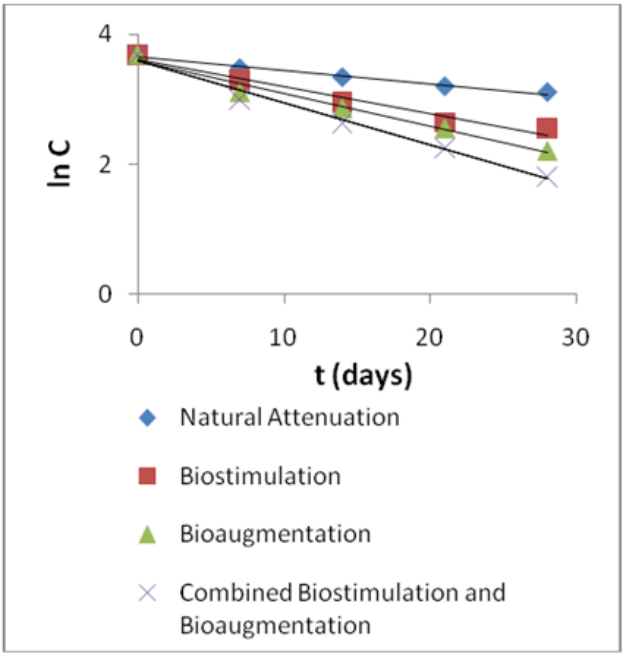

Figure 3. First-order kinetic model equation fitted to the naphthalene biodegradation data under (a) natural attenuation, (b) biostimulation, (c) bioaugmentation, and (d) combined biostimulation and bioaugmentation

4. Conclusion

From this present study, it can be concluded that the reduction of naphthalene in the contaminated soil indicates the presence of PAH-degrading microbial communities; and that the rate of naphthalene biodegradation in soil could be enhanced by the addition of inorganic nutrients and inoculum, respectively. The soil treatment under combined biostimulation and bioaugmentation exhibited the highest degree of biodegradation with the highest biodegradation rate constant (k = 0.064 day^{-1}) and lowest half-life time ($t_{1/2}$ =10.8 days)) and the soil treatment under natural attenuation the least degradation with the lowest biodegradation rate constant (k = 0.020 day^{-1}) and highest half-life time ($t_{1/2}$ = 34.7 days). Thus, the use of biostimulation and bioaugmentation to enhance naphthalene biodegradation in the soil could be one of the severally sought bioremediation strategies of remediating natural ecosystem (environment) contaminated with petroleum hydrocarbons.

References

[1] Bisht, S., Pandey, P., Sood, A., Sharma, S., Bisht, N.S. "Biodegradation of naphthalene and anthracene by chemo-tactically active rhizobacteria of *populus deltoids*". *Brazilian Journal of Microbiology* 41: 922-930, 2010.

[2] Miya, R.K., Firestone, M.K. "Phenanthrene-degrader community dynamics in rhizosphere soil from a common annual grass". *Journal of Environmental Quality* 29: 584-592, 2000.

[3] Directive 2000/60/EC, 2000. EU Water Framework Directive. Official Journal L 327.

[4] U.S. Environmental Protection Agency Technical Background Document to Support Rulemaking Pursuant to the Clean Air Act - Section 112(g). Ranking of Pollutants with Respect to Hazard to Human. EPA-450/3-92-010.Emissions Standards Division, Office of Air Quality Planning and Standards, Research Triangle Park, NC.1994.

[5] Mueller J. G., Chapman P.J., Pritchard P.H. "Creosote-contaminated sites". *Environmental Science and Technology* 23: 1197-1202, 1989.

[6] Siciliano, S.D., Germida, J.J., Banks, K., Greer, C.W. "Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial". *Applied Environmental Microbiology* 69: 483-489, 2003.

[7] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Naphthalene and 2-Methylnaphthalene. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, 1990.

[8] Amoore J. E., Hautala E. "Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution". *Journal of Applied Toxicology* 3: 272-290, 1983.

[9] Grund, Denecke B, Eichenlab "Naphthalene degradation via salicylate and gentisate by *Rhodococcus spp*.strain B4" *Applied Environmental Microbiology* 55:1874-1877, 1992.

[10] Karl J Rockne, Joanne C Chee-Sanford, Robert A Sanford, Brian P Hedlund, James T Stanley, et al. "Anaerobic Naphthalene Degradation by Microbial Pure Cultures under Nitrate-Reducing Conditions". *Applied and Environmental Microbiology* 66: 1595-1601, 2000.

[11] Sukor, M.Z., Yin, C-Y, Savory, R.M., Abdul-Talib, S. "Biodegradation kinetics of naphthalene in soil medium using Pleurotus ostreatus in batch mode with addition of fibrous biomass as a nutrient". *Bioremediation Journal*, 16 (3): 177-184, 2012.

[12] Pawar AN, Ugale SS, More MG, Kokani NF, Khandelwal SR "Biological Degradation of Naphthalene: A New Era". *Journal of Bioremediation and Biodegradation* 4: 203.2013

[13] Bach, Q.D., Kim, S.J., Oh, Y.S. "Enhancing the intrinsic bioremediation of PAH-contaminated anoxic estuarine sediments with biostimulating agents". *Journal of Microbiology* 43(4): 319-324, 2005.

[14] Owabor, C.N., Ogunbor, O.F. "Naphthalene and pyrene degradation in contaminated soil as a function of the variation of particle size and percent organic matter". *African Journal of Biotechnology* 6(4): 436-440, 2007.

[15] Agarry, S. E., Yusuf, R. O., Ajani, A. O. "Biodegradation of anthracene and naphthalene in soil". *Journal of Nigerian Society of Chemical Engineers*, 25 (1-2): 55-63, 2010a.

[16] Agarry, S.E., Owabor, C.N. "Anaerobic bioremediation of marine sediment artificially contaminated with anthracene and naphthalene". *Environmental Technology*, 32(12): 1375-1381, 2011.

[17] Kanaly, R.A., Harayahama, S. (2000) Biodegradation of high molecular weight polycyclicaromatic hydrocarbons by bacteria. J. Bacteriol. 182: 2059-2067.

[18] Han, M.j., Choi, H.T., Song, H.G. "Degradation of phenanthrene by *Trametes versicolor* and its laccase". *Journal of Microbiology* 42: 94-98, 2003.

[19] Thomas, S.P., Stensel, H.D., Strand, S.E. "Biodegradation of polyaromatic hydrocarbons by marine bacteria Effect of solid phase on degradation kinetics". *Water Research*. 33: 868-880, 1998.

[20] Elliot, M. "*Polycyclic aromatic hydrocarbons and redox parameter in a creosote-contaminated aquifer*". M.Sc. Thesis, Virginia Polytechnic Institute and State University, USA, 2001.

[21] Wang, X.C., Zhang, Y.X., Chen, R.F. "*Distribution and partitioning of polycyclic aromatic hydrocarbons (PAHs) in different size fractions in sediments from Boston Harbor, United States*", 2001.

[22] Leahy, J.G., Colwell, R.R. "Microbial degradation of hydrocarbons in the environment". *Microbiological Reviews* 54: 305-315, 1990.

[23] Thibault, S.I., Anderson, M., Frankenberger, W.T. "Influence of surfactants on pyrene desorption and degradation in soil". *Applied Environmental Microbiology* 62(1): 283-287, 1996.

[24] Agarry SE, Owabor CN, Yusuf RO "Enhanced bioremediation of soil artificially contaminated with kerosene: Optimization of biostimulation agents through statistical experimental design". *Journal of Petroleum and Environmental Biotechnology* 3:120, 2012.

[25] Vogel, T.M. "Bioaugmentation as a soil bioremediation approach". *Current Opinion in Biotechnology* 7: 311-316, 1996.

[26] Berardesco G, Dyhrman S, Gallagher E, Shiaris MP "Spatial and temporal variation of phenanthrene-degrading bacteriain intertidal sediments". *Applied and Environmental Microbiology* 64: 2560-2565, 1998.

[27] McLean, E. O. Soil pH and lime requirement In "*Methods in Soil Analysis: Chemical and Microbiological Properties"*, Part II, ed. C. A. Black. Madison, WI: American Society of Agronomy, 1982.

[28] Nelson, D. W., Sommers. L.E. Determination of organic carbon. In "*Method of Soil Analysis"*, vol. 2, ed. A. L. Page, R. H. Miller, and D. R. Keeney, 539. Madison, WI: American Society of Agronomy, 1982.

[29] Bremner, J. M., Mulvaney, C.S. Total nitrogen determination. In "*Method of Soil Analysis"*, vol. 2, ed. A. L. Page, R. H. Miller, and D. R. Keeney, pp. 595. Madison, WI: American Society of Agronomy, 1982.

[30] Olsen, S. R., Sommers. L.E. Determination of available phosphorus. In "*Method of Soil Analysis"*, vol. 2, ed. A. L. Page, R. H. Miller, and D. R. Keeney, 403. Madison, WI: American Society of Agronomy, 1982.

[31] Amanchukwu, C. C., Obafemi, A., Okpokwasili, G.C. Hydrocarbon degradation and utilization by a palmwine yeast isolate. FEMS Microbiol. Lett. 57:51-54, 1989.

[32] APHA "*Standard Methods for the Examination of Water and Wastewater"*, 21st ed., American Public Health Association, Washington, DC, 2005.

[33] Krieg, N. R., Holt, J. G., Sneath, P. H.A., Stanley, J. T., and Williams, S. T. "*Bergey's Manual of Determinative Bacteriology"*, 9th ed., Williams and Wilkins, Baltimore, 1994.

[34] Agarry, S. E., Owabor, C. N., Yusuf, R. O. "Studies on biodegradation of kerosene in soil under different bioremediation strategies". *Bioremediation Journal.* 14(3): 135-141, 2010b.

[35] Beolchini F, Rocchetti L, Regoli F, Dell'Anno A "Bioremediation of marine sediments contaminated by hydrocarbons: Experimental analysis and kinetic modeling". *Journal of Hazardous Material* 182: 403-407, 2010.

[36] Olabemiwo, OM., Adediran, GO., Adekola, FA., Adelowo, OO., Olajire, AA "Biodegradation of hydrocarbon compounds in Agbabu natural bitumen". *African Journal of Biotechnology*, 13 (11): 1257-1264, 2014.

[37] Zhu X, Venosa AD, Suidan MT, Lee K "*Guidelines for the Bioremediation of Marine Shorelines and Freshwaters Waterlands*". US Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory, 2001.

[38] Wang, JL., Han, L.P., Shi, H.C., Qian, Y. **"**Biodegradation of quinoline by gel immobilized *Burkholderia* sp". *Chemosphere* 44:1041-1046, 2001.

[39] Greene, EA., Kay, JG., Jaber, K., Stehmeier, LG., Voordouw, G "Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons". *Applied and Environmental Microbiology* 66, 5282-5289, 2000.

[40] Aronson, D., Boethling, R., Howard, P., Stiteler, W (2006) Estimating biodegradation half-lives for use in chemical screening. Chemosphere 63: 1953-1960 (2006).

[41] Sinkkonen, S., Paasivirta, J "Degradation half-life times of PCDDs, PCDF sand PCBs for environmental fate modeling". *Chemosphere* 40, 943-949, 2000.

[42] Dimitrov, S., Pavlov, T., Nedelcheva, D., Reuschenbach, P., Silvani, M. et al. "A kinetic model for predicting biodegradation". *SAR QSAR Environmental Research* 18, 443-457, 2007.

[43] Matthies, M., Witt, J., Klasmeier, J (2008) Determination of soil biodegradation half lives from simulation testing under aerobic laboratory conditions: a kinetic model approach. *Environmental Pollution* 156, 99-105, 2008.

[44] Zahed, MA, Abdul Aziz, H., Isa, MH, Mohajeri, L., Mohajeri, S., Kutty, SRM. "Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500". *Journal of Hazardous Materials* 185, 1027-1031, 2011.

[45] Agarry, SE, Aremu, MO., Aworanti, OA. (2013) Kinetic modelling and half-life study on bioremediation of soil co-contaminated with lubricating motor oil and lead using different bioremediation strategies. *Soil and Sediment Contamination- An International Journal* 22 (7), 800-816, 2013.

[46] Yeung, PY., Johnson, RL., Xu, JG. "Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration". *Journal of Environmental Quality* 26, 1511-1576, 1997.

[47] Shabir, G., Afzal, M., Anwar, F., Tahseen, R., Khalid, Z.M. "Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrient conditions". *International Journal of Biodeterioration and Biodegradation* 61, 161-166, 2008.

[48] Abdulsalam, S., Omale, A.B. "Comparison of biostimulation and bioaugmentation techniques for the remediation of used motor oil contaminated soil". *Brazilian Archives of Biology and Technology* 52, 747-754, 2009.

[49] Abdulsalam, S., Bugaje, I. M., Adefila, S. S., Ibrahim, S. (2011) Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. *International Journal of Environmental Science and Technology* 8(1), 187-194, 2011.

[50] Bento, F. M., Camargo, F.A., Okeke, B., Frankenberger, Jr. T.W. (2003) Bioremediation of soil contaminated by diesel oil. *Brazilian Journal of Microbiology* 34(Suppl. 1), 65-68, 2003.

[51] Xu, Y., Lu, M. "Bioremediation of crude oil-contaminated soil: comparison of different biostimulation and bioaugmentation treatments". *Journal of Hazardous Materials* 183 (1-3), 395-401, 2010.