pH and Sulphate mass: Intensity factors in investigating sulphate-dependent degradation of polycyclic aromatic hydrocarbons

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ABSTRACT

The ability of pH and sulphate masses in influencing the rate of degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soil leachate was studied. Results obtained indicated that using 2 g sodium sulphate (Na_2SO_4) about 14.0 % of 2-methylnaphthalene was degraded as the highest PAH at pH 4.0, while anthracene (1.1 %) was the least degraded PAH. However, using 4 g Na_2SO_4, 11.5 % of 2-methylnaphthalene was the highest degraded PAH, while fluoranthrene and pyrene were not detected. It was also found that a trend in PAHs degradation was observed as the pH was increased from 2.0, 4.0 and 6.0 using 2 g Na_2SO_4. Nevertheless, this trend in PAHs degradation was not replicated using 4 g Na_2SO_4. The two-ring PAHs showed high degradation potential using both Na_2SO_4 masses. However, it was revealed that increasing the weight of Na_2SO_4 from 2 to 4 g had no notable effect in PAHs degradation.

Keywords: chemical, fluoranthrene, hydrocarbon, leachate, remediation, substrate, surfactant.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and consistently present in the environment. They are typically formed during the incomplete burning of organic material including wood, coal, oil, gasoline and garbage. PAHs are also associated with human activities such as cooking, heating homes and industries, and fuel for operating automobiles, although low levels of PAHs are also present in the environment from natural sources, such as forest fires. Their presence in the environment at higher concentrations is a factor of habitation and is due to widespread practice of emptying fireplaces, stoves, boilers, garbage etc. into the environment in rural and urban areas over the past centuries. Hence, PAHs are commonly found in soils. Volcanic activity and biosynthesis by bacteria and plants are other natural sources of PAHs. Relative to fires, these sources contribute small amounts to the environment.

PAHs enter the environment mostly as releases to air from volcanoes, forest fires, residential wood burning and exhaust from automobiles and trucks. They can also enter surface water through discharges from industrial plants and wastewater treatment plants, and can be released to soils at hazardous waste sites if they escape from storage containers. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water, and how easily they evaporate into the air. PAHs can be degraded under aerobic or anaerobic conditions. The ability of aerobic microorganism to degrade PAHs such as naphthalene, anthracene, biphenyl, and benzo(a)pyrene has been well documented [1],[2],[3]. Several studies have suggested that some PAHs can be degraded anaerobically if nitrate is available as electron acceptor [4],[5]. In organic matter degradation, sulphate reduction and in some instances, Fe (III) and Mn (IV) reduction have been reported to be the dominant process of degradation [6].

1132

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However, in a recent study, it was observed that mono and polycyclic aromatic hydrocarbons were rapidly oxidized to CO_2 with the reduction of sulphate in petroleum-contaminated sediments [7]. The study demonstrated that ^{14}C naphthalene and phenanthrene were also oxidized to $^{14}CO_2$ without a detectable lag under strict anaerobic conditions in sediments that were heavily contaminated with PAHs. It was shown that sulphate reduction was necessary for the oxidation of the PAHs [6]. The influence of anaerobic conditions on aqueous-phase polycyclic aromatic degradation (PAH) bioavailabilty was investigated in laboratory microcosms by Pravecek et al.[8]. Highly aged PAH-contaminated soil was incubated under anaerobic conditions by using various anaerobic headspaces, namely; anaerobic headspaces with an oxygen-scavenging complex [titanium (III) citrate] in the aqueous phase and anaerobic headspaces with electron-acceptor amendments in the aqueous phase. Incubation of soil solely under anaerobic conditions resulted in increased aqueous concentrations of all PAHs tested (fluoranthrene, pyrene, benzo(a)anthracene, and benzo(a)pyrene. The degree of solubility increase observed was a function of molecular weight of the PAHs regardless of initial soil concentrations, suggesting formation of stable PAH-soluble organic matter associations. Another microcosm study has been conducted to address the influences of air-soil partition and sequestration on the fate of PAHs in soil. Sterilized and unsterilized soils with soil organic carbon (SOC) content ranging from 0.23 to 7.06 % were incubated in a chamber with six PAHs supplied through air. After 100 days of incubation, when the system had approached pseudo-steady state, the PAHs concentrations in the unsterilized soils still correlated with SOC significantly, while the association organic matter associations did not exist for those sterilized. The lower degradation rate in the soil with higher SOC was likely the major reason for the association between SOC and PAHs concentrations, while the decrease surface porosity likely suppressed such correlation for the sterilized samples. The results indicated that the sequestration was likely the major mechanism for the accumulation of PAHs in soil since both soil porosity and PAHs property had observed influences on the system [9]. A study investigating anaerobic degradation of five PAHs from Erren river sediment in southern Taiwan noted that the degradation rates of PAH were in the order: acenaphthene>fluorine.phenanthrene>anthracene>pyrene. The degradation rate was enhanced when the five compounds were present simultaneously in the river sediment [10].

The efficiency of PAHs biodegradation is limited because these compounds have very low aqueous solubility and vapor pressure [11]. Surfactants have been shown to be useful for bioremediation of sites polluted with PAHs in overcoming the problems associated with low PAHs solubility since they enhance the solubility of hydrophobic compounds [12]. Many studies have stressed the importance of surfactants to increase the solubility of PAHs by decreasing the interfacial surface tension between PAHs and the soil/water interphase [13]. When surfactant concentration is above the critical micelle concentration, micelle aggregates provide an additional hydrophobic area in the central region of micelles thus enhancing the aqueous solubility of PAHs. In a recent study, the effect of several non-ionic surfactants (Tween-80, Triton X-100, and Treginol NP-100) on the ability of different bacteria (*Enterobacter* sp., *Pseudomonas* sp., and *Stenotrophomonas* sp.) to degrade PAHs was evaluated. Bacterial cultures were performed at 25 ^{o}C in an orbital shaker in medium containing 1 % of surfactant and 500 mg/l of each PAH. Result obtained indicated that experiments performed with Tween-80 gave the highest cell density values and maximum specific growth rate because the surfactant was used as a carbon source by all bacteria. Triton X-100 and Tergitol NP-100 were not biodegraded. PAHs degradation rate was higher especially by the action of *Enterobacter* sp. With Tween-80 and Triton X-100. Control experiments performed without surfactant showed a significant decrease in biomass growth rate with a subsequent loss of biodegradation activity likely due to a reduced solubility and bioavailability of PAHs in absence of surfactant [14]. Efficiency of surfactant-enhanced desorption for contaminated soils depend on the component characteristics of soil-surfactant-PAHs system. Zhou & Zhu [15] revealed that surfactants enhance PAHs desorption only when the relative efficiency coefficient value is larger than 1 and the added surfactant concentration greater than the corresponding critical enhance desorption concentration. This fact thus highlight the point that efficiency of surfactants in enhancing PAH desorption show strong dependence on the soil composition, surfactant structure, and PAH properties; and further demonstrate practical interest for the selection of surfactants to optimize soil remediation technologies.

In the present study, the effect of pH and Na_2SO_4 mass as intensity factor in evaluating sulphate-dependent degradation of PAHs in contaminated soil leachate was investigated.

MATERIALS AND METHODS

The soil sample used in this experiment was obtained from the commercial bus park of the Federal University of Technology, Owerri, Nigeria. Soil pre-treatment include sieving to select particle size of 10 mm and air dried for 48 hrs. Obtaining this particle size is important to facilitate contact between soil and contaminant as well a organic

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solvent used for extraction. The soil sample was dried in an autoclave (Ac 064) for 30 mins at 105 ^{o}C. About 5 kg of the dried soil sample was placed into a 25 L plastic bucket previously washed and rinsed with deionised water. About 500 g of the dried soil sample was also separately weighed and placed in a 1 L beaker. This sample in the 1 L beaker was used for the control experiment.

About 5 L of waste automobile engine oil was poured into the soil sample in the 25 L plastic bucket. It has been previously reported that waste automobile engine oil contain 2-6 ring PAH compounds [16],[17]. The mixture was triturated for 10 mins and 5 L of n-hexane solution was added to the triturated mixture and further trituration was performed for another 10 mins. About 10 L of distilled water was then added to the mixture and the content was stirred for a further 10 mins and allowed to stand for 2 hrs. The supernatant was decanted and filtered through Whatman No. 42 filter papers. About 9 L of the filtrate was recovered to serve as the stock solution.

To 100 ml of the stock solution in a 250 ml beaker was added 2 g of Na_2SO_4 powder. The mixture was stirred for 2 mins and the pH of the mixture was adjusted to 2.0 using 1 M NaOH/HCl as appropriate and filtered. The filtrate was then transferred into a 500 ml separatory funnel and 500 ml of n-hexane solution was further added. The mixture was shaken for 2 mins with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase and collected through a funnel containing solvent-moistened filter paper containing anhydrous sodium sulphate into a 1 L Erlenmeyer flask. The solvent was evaporated on a water bath at 110 ^{o}C to 10 ml and concentrated with a stream of nitrogen gas to 2 ml.

PAHs concentration in the extract was determined using Gas Chromatography/Mass Spectroscopy (GC/MS), Shimadzu QP2010 GCMS, Japan, at flow rate 1.18 ml/min with a helium carrier gas, column oven temperature was ramped at 80 to 280 ^{o}C at 5 ^{o}C/min with 5 min holding time, then to 300 ^{o}C at 10 ^{o}C/min with 10 min holding time. HP5MS column (30m x 0.25μm x 0.25 mm ID) was used. Three replicate sample treatments were processed and mean PAHs concentration was obtained.

The above procedure was repeated at pH 4.0, 6.0 and 8.0 respectively. However, the procedure was also repeated at the above pH range (2.0, 4.0, 6.0 and 8.0) using 4 g Na_2SO_4.

The control sample was also processed as in above at pH 2.0 without addition of Na_2SO_4.

3.0 Statistical Analysis
Data are given as arithmetic mean and standard deviation. The *F*-test was used to estimate significant difference in mean PAHs concentration between pH levels.

RESULTS AND DISCUSSION

Table 1 show values of PAHs in μg/g of soil leachate using 2 g Na_2SO_4 at pH 2.0, 4.0, 6.0, and 8.0 respectively. It was found that high degradation rate was observed with the two-ring PAHs compounds (naphthalene and 2-methylnaphthalene). 2-methylnaphthalene was the highest PAH degraded overall averaging 14.0 % at pH 4.0. Anthracene was the least degraded PAH overall totaling 1.1 % at pH 2.0, 4.0 and 6.0 respectively. It was also noted that there was a general trend in PAHs degradation as the pH was exceeded from 2.0 to 4.0. However, there was a sharp decrease in PAHs degradation at pH 6.0. But as the pH was increased from 6.0 to 8.0, increase in PAHs degradation was not statistically significant, since the *F*-test value between pH 6.0 and 8.0 was obtained as 2.77. Testing this value at 8 and 8 degree of freedom, $P < 0.05$.

However, **Table 2** also show values of PAHs in μg/g of soil leachate using 4 g Na_2SO_4 at pH 2.0, 4.0, 6.0, and 8.0 respectively. Two-ring PAHs were also remarkably degraded. About 9.6 % of naphthalene was degraded at pH 8.0, while 11.5 % of 2-methylnaphthalene was the overall highest PAH degraded at pH 8.0. Fluoranthrene and pyrene were not detected at pH 4.0 and 6.0. Degradation of PAHs using 4 g Na_2SO_4 was skewed as the pH was increased. Meanwhile, there increase in PAHs degradation as the pH was increased from 4.0 to 6.0 was not statistically significantly different, also because, $P < 0.05$ when the *F*-test value (1.56) of PAHs degradation between pH 4.0 and 6.0 was tested at 8 and 8 degree of freedom. It was also observed that increasing the mass of Na_2SO_4 from 2 to 4 g had no notable effect on PAHs degradation. For instance, the *F*-test value of PAHs degradation between 2 g Na_2SO_4 at pH 2.0 and 4 g Na_2SO_4 also at pH 2.0 is 1.89. Testing this value at 8 and 8 degree of freedom $P < 0.05$.

Table 1: Values of PAHs in μg/g of soil leachate using 2 g Na$_2$SO$_4$ at various pH range

PAHs	pH 2.0[i] Mean (μg/g) ± SD	pH 4.0[ii] Mean (μg/g) ± SD	pH 6.0[iii] Mean (μg/g) ± SD	pH 8.0[iv] Mean (μg/g) ± SD	Control Mean (μg/g) ± SD
Naphthalene	15.116 ± 0.1	18.559 ± 0.1	12.063 ± 0.2	12.119 ± 0.5	143.9 ± 0.1
2-methyl naphthalene	11.092 ± 0.2	13.925 ± 0.4	9.042 ± 0.2	9.828 ± 0.4	96.41 ± 1.2
Acenaphthylene	0.165 ± 0.2	0.195 ± 0.5	0.145 ± 0.2	0.346 ± 0.6	12.98 ± 1.2
Acenaphthrene	0.157 ± 0.2	0.185 ± 0.3	0.136 ± 0.1	0.223 ± 0.1	10.66 ± 0.1
Fluorene	0.212 ± 0.1	0.268 ± 0.2	0.187 ± 0.0	0.452 ± 0.1	6.937 ± 0.3
Phenanthrene	0.473 ± 0.3	0.584 ± 0.1	0.410 ± 0.1	0.594 ± 0.3	5.993 ± 1.1
Anthracene	0.076 ± 0.2	0.075 ± 0.0	0.073 ± 0.0	0.189 ± 0.2	6.728 ± 1.4
Fluoranthrene	0.110 ± 0.1	0.126 ± 0.2	0.106 ± 0.1	0.207 ± 0.2	3.625 ± 1.4
Pyrene	0.137 ± 0.2	0.165 ± 0.1	0.124 ± 0.6	0.219 ± 0.1	4.921 ± 0.6

F-test; iii and iv = 2.77

Table 2: Values of PAHs in μg/g of soil leachate using 4 g Na$_2$SO$_4$ at various pH range

PAHs	pH 2.0[a] Mean (μg/g) ± SD	pH 4.0[b] Mean (μg/g) ± SD	pH 6.0[c] Mean (μg/g) ± SD	pH 8.0[d] Mean (μg/g) ± SD	Control Mean (μg/g) ± SD
Naphthalene	10.059 ± 0.8	0.438 ± 0.2	0.223 ± 0.1	13.786 ± 0.7	143.9 ± 0.1
2-methyl naphthalene	8.972 ± 0.6	0.321 ± 0.2	0.306 ± 0.1	11.091 ± 0.5	96.41 ± 1.2
Acenaphthylene	0.330 ± 0.1	0.145 ± 0.1	0.145 ± 0.2	0.165 ± 0.1	12.98 ± 1.2
Acenaphthrene	0.216 ± 0.1	0.138 ± 0.1	0.138 ± 0.1	0.243 ± 0.1	10.66 ± 0.1
Fluorene	0.421 ± 0.1	0.144 ± 0.1	0.144 ± 0.1	0.152 ± 0.1	6.937 ± 0.3
Phenanthrene	0.560 ± 0.1	0.158 ± 0.1	0.158 ± 0.2	0.150 ± 0.1	5.993 ± 1.1
Anthracene	0.184 ± 0.2	0.144 ± 0.1	0.144 ± 0.2	0.052 ± 0.0	6.728 ± 1.4
Fluoranthrene	0.204 ± 0.1	ND	ND	0.040 ± 0.0	3.625 ± 1.4
Pyrene	0.212 ± 0.1	ND	ND	0.194 ± 0.1	4.921 ± 0.6

F-test; b and c = 1.56
ND = Not Detected

Until recently, sulphate reduction was not considered a significant remediation pathway because it was of insufficient energy to cleave fused aromatic rings [18]. However, recent evidence has shown that long-term exposure of substrate to contaminants is a factor to examine the capacity of microorganisms to biodegrade PAHs under sulphate-reducing conditions [19]. Lovley et al., [7], has shown that degradation of single-ring aromatics by sulphate-reducing organism could be achieved if sediments are exposed to PAHs for many years. Another study by Rockne & Strand [20] also showed that the degradation of naphthalene and phenanthrene by sulphate-reducers resulted in stoichiometric sulphide production when the contaminated sediments have been exposed to long-term contamination by PAHs. According to Kennedy et al., [21], measurement of sulphate-reduction should include process factors other than aqueous parameters since aqueous parameters are not a conclusive means of quantifying sulphate-reduction of PAHs. Generally, PAHs degradation rate in natural systems is inversely related to the number of fused aromatic rings in the compound. This is an important factor in facilitating the remediation of low molecular weight (LMW) PAHs. LMW PAHs have high vapor pressure making volatilization an important remediation pathway and hence high selective degradation potential [22].

PAHs fate under anaerobic condition depends not only on substrate interactions and composition of microbial population but also on pH and redox potential conditions as well as temperature and salinity [23]. Temperature may also affect PAHs degradation through its action on bioavailability. It has been found that low temperature mainly affected degradation of less soluble, larger PAHs having three or more aromatic rings [24]. The impact of salinity on PAHs degradation in estuarine sediments has also been studied [25]. An important factor for successful PAHs degradation activity is the pH of the soil. Kästner et al.,[26] had noted that small pH shifts have dramatic effects on the degradation of low concentration hydrocarbons. Hence, environmental conditions in soil need to be adjusted carefully if the full potential of pH effect on redox chemicals in degradation of PAHs in soil must be achieved.

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CONCLUSION

The present study noted a characteristic change in PAHs degradation in individual PAH especially with the two-ring PAHs. Based on this line of observation, the study would encourage further research to adequately establish the mode of degradation of two-ring PAHs using chemical reduction processes.

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