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Full Length Research Paper

Naphthalene and pyrene degradation in contaminated soil as a function of the variation of particle size and percent organic matter

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The effect of soil particle size distribution and percent organic matter on the degradation rate of naphthalene and pyrene in a water medium of 7.05 ml/min at 27 ± 2^oC in a soil reactor was studied. Analysis of the pattern of disappearance of these polycyclic aromatic hydrocarbons (PAHs) using various particle sizes showed a rapid decline of concentration of the chemicals during initial stages of bioremediation treatment, followed by a slow reduction rate. The extent of naphthalene and pyrene removal and final concentrations for the period under study differed among the different soil particle sizes. Results show that from an initial 100 mg/l, the concentration of both naphthalene and pyrene decreased in the following sequence clay > silt > fine sand > coarse sand. The degradation of the two PAHs was significantly enhanced by the addition of organic matter to the bulk composite soil. The specific reaction rate constant k was found to increase with decreasing particle size and increase with increasing % organic matter. For both PAHs, coarse sand had the lowest rate constant while clay had the highest. This implies that degradation was faster in the clay fraction than in the other soil fractions. The correlation coefficients obtained using linear regression method was between 0.734 and 0.996 indicating the reliability of the experimental data.

Key words: Naphthalene, pyrene, biodegradation, particle size, organic matter, contaminated soil.

INTRODUCTION

Man has dealt with the clean up of petroleum products contamination almost since the first day oil was discovered. Around the beginning of the 1990's, the spilling of more than 20,000 barrels of crude oil from the tanker Exxon Valdez, Alaska as well as smaller spills in Texas refocused attention on the problem of hydrocarbon contamination of the environment (Hagar, 1989). The potential for bioremediation as a biological treatment technique for the clean up of contaminated soils and sediments is almost boundless.

In Nigeria, many private and governmental facilities are faced with the clean up arising from either accidental or purposeful spill of petroleum and petroleum products.

One important group of petroleum hydrocarbons, which constantly pollute the soil and aquatic environment today, is the polycyclic aromatic hydrocarbons (PAHs). These groups of chemicals are priority pollutants due to their lipophilic nature (Bewley et al., 1989). They have the potential to bind to particulates in soil and sediments rendering them less available for microbial uptake and their ability to degrade in soils has become a major concern to environmental researchers (Boonchan et al., 2000).

The overall degradation rates of hydrocarbons biodegradation in soils are strictly limited by a variety of parameters (Rockne et al., 2002). It is therefore necessary to understand the factors limiting microbial degradation in order to adopt appropriate methodology to optimize the process of degradation. Documented reports on the various conditions and factors, which determine the rate of degradation of PAHs, abound (McGill, 1980; Atlas, 1981; Sandrik et al., 1986; Block et al., 1993; Oles-

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zczuk and Baran, 2003).

The focus of this study is to evaluate the level of mineralization of naphthalene and pyrene that can be achieved over time within various particle sizes of the soil matrix. The degradation profile due to the utilization of these compounds as carbon and energy sources under the influence of varying soil organic matter content is also under investigation.

MATERIALS AND METHODS

Soil samples were collected from the unimpacted zones of the Nigerian Institute For Oil Palm Research (NIFOR). About 2 kg of bulk surface and subsurface soil was collected from different points on the sampling sites. The bulked composite soil was preserved by putting them in sterile polyethylene bag properly sealed before taking them to the laboratory for analysis. The soil still sealed in the polyethylene bags were put in the refrigerator prior to the batch degradation study.

Particle size distribution

Mechanical analysis of the soil was carried out by the hydrometer method after the destruction of organic matter with hydrogen peroxide (Day, 1965). 50 g of the air dried soil was weighed into a 600 ml beaker. 50 ml of distilled water was added followed by 10 ml of 30 ml volume H_2O_2. The suspension was heated on a hot plate until frothing stopped and the liquid almost dried. The soil was then transferred into a milk shake cup with baffles. 100 ml of water and 20 ml of 20% sodium hexametaphosphate solution (algon) was added to the cup and the suspension stirred for 30 min.

The suspension was then transferred into a 1 litre, measuring cylinder with a jet of water from wash bottle and the volume brought to mark with distilled water. The suspension was agitated vigorously to ensure thorough mixing and exactly 40 s from when agitation stopped, a hydrometer reading was taken and the temperature of the suspension also recorded.

The suspension was left undisturbed on the bench. At the end of 24 h, another hydrometer and temperature readings were taken. The various particle sizes were calculated as below:

$$\% \text{ Sand} = 100 - \text{Corrected 40 s hydrometer reading} \times \frac{100}{50}$$

$$\% \text{ Clay} = \text{Corrected 2 h hydrometer reading} \times \frac{100}{50}$$

$$\% \text{ Silt} = 100 - (\% \text{ Sand} + \% \text{ Clay})$$

pH

The hydrogen ion concentration of the soil was determined using a corning (model 260T) glass electrode pH meter. 30 g of 2 mm air dried sample was weighed into a 100 ml beaker and 30 ml of distilled water was added. The suspension was stirred intermittently for 30 min and the pH was measured using a glass electrode pH meter after the meter had been standardized with pH buffer 4.0 and 7.0 solutions.

Organic carbon

Organic carbon content of the soil was determined by the chromic acid wet oxidation procedure as described by Black, 1965.

Organic matter

This was determined by multiplying the % organic carbon value by a factor of 1.724.

Total nitrogen

The total N content of the soil was extracted by the micro Kjeldahl procedure and the ammonium in the extract assayed by the alkaline- phenate colorimetric method (Fiore and O'Brien, 1968).

Preparation of stock samples extraction and analysis

100 mg of pyrene crystals was dissolved in 100 ml of methanol in a measuring cylinder and the mixture was stirred continuously until the crystals dissolved. The solution was then introduced into 100 g of the already prepared portions of fractionated and bulked soil samples. The same procedure was carried out to dissolve the naphthalene crystals using equal amount as that of pyrene.

The experimental soil samples were monitored under room temperature of 27 $\pm$ 2°C. Oxygen was supplied at a rate of 2 cm^3/min to ensure adequate aeration of the soil column. Sampling for quantitative analysis was carried out on a weekly basis. Extraction using n-hexane was first performed to release the contaminants from the soil matrix. The hexane extracts were each evaporated to 2 ml in a water bath.

The samples for analysis was collected from experimental set up with the help of sterile syringes and transferred into sample storage bottles. They were kept in the refrigerator prior to analysis. This preservation was to prevent further microbial degradation before analysis. Gas chromatograph analysis was carried out by the Hewlett Packard model 5890 series 11, equipped with flame ionization detector. The column temperature was 40 - 300°C, programmed at 100°C/min. Nitrogen was used as a carrier gas, at a pressure 60 – 65 psi. The pressure of hydrogen and air are 35 and 40 psi, respectively, with injector and detector temperatures 25 and 32°C respectively.

RESULTS AND DISCUSSION

The main objective of this study was to provide an insight on the degradation pattern using soil of varying particle sizes and percent organic matter. The results of the physicochemical analysis on the soil used to conduct the biodegradation experiment are shown below in Table 1. The dominant particle size was between 0.35 - 0.5 mm with fewer than 5% greater than 2.0 mm. This suggests high porosity. A pH value of 4.72, which implies a reducing environment, was obtained. Oxygen was used to bring about a redox condition, which promotes micro-bial activity. The soil was characterized by low total nitro-gen suggesting low nutrient level. This was however, boosted by supplements as prescribed by the organi-zation for economic development and cooperation.

The results of the batch mineralization of naphthalene and pyrene using different soil particle sizes and bulked soils are shown in Tables 2 and 3. The influence of particle size on degradation depicted that the concentration of naphthalene decreased from an initial 100 mg/g soil to 20, 12, 10, and 2.25 mg/g for coarse sand, fine

Table 1. Physicochemical analysis on unimpacted soil.

Parameters	Value
Total Nitrogen	0.03%
Soil Organic Carbon Content	0.47%
% Silt	19
% Clay	26
% Coarse Sand	21
% Fine Sand	34
pH	4.72
% organic matter in 100 g cow dung	4.2

Table 2. Concentration profile for naphthalene in different soil fractions.

Week	Coarse sand (0.25 – 2 mm) (mg/g soil)	Fine sand (0.02 - 0.25 mm) (mg/g soil)	Silt (0.002 - 0.02 mm) (mg/g soil)	Clay (<0.002 mm) (mg/g soil)	Bulked soil (mg/g soil)
0	100	100	100	100	100
1	42.21	37.75	26.18	19.54	23.78
2	40.23	30.20	24.12	15.25	20.65
3	34.50	26.10	20.00	10.35	18.40
4	29.00	20.06	15.12	6.36	15.20
5	20.00	12.00	10.00	2.25	10.85

Table 3. Concentration profile for naphthalene in different soil fractions.

Week	Coarse sand (0.25 – 2 mm) (mg/g soil)	Fine sand (0.02 - 0.25 mm) (mg/g soil)	Silt (0.002 - 0.02 mm) (mg/g soil)	Clay (<0.002 mm) (mg/g soil)	Bulked soil (mg/g soil)
0	100	100	100	100	100
1	84.33	54.20	43.84	18.97	33.07
2	22.80	48.10	40.15	17.00	32.00
3	78.45	42.10	32.00	15.75	30.15
4	60.80	33.10	27.12	12.06	25.80
5	48.00	20.50	20.00	7.00	22.40

sand, silt and clay, respectively. Similarly, a decline to 48, 20.5, 20.0 and 7 mg/g for for coarse sand, fine sand, silt and clay, respectively, was also obtained for pyrene. These results suggest that the biodegradation of naphthalene and pyrene follow the same trend in the soil fractions except that comparatively, the degree of degradation was consistently higher in naphthalene. This phenolmenon can be attributable to the fact that low molecular weights PAH degrade faster than those of higher molecular weight. The more the number of rings, the less soluble they are in aqueous solutions.

Tables 4 and 5 illustrate the biodegradation curves for naphthalene and pyrene in bulked soil without organic matter content and with different percent organic matter content. Biodegradation increased from 0 - 4% organic matter, thereby leading to a decrease in concentration from the initial 100 mg/g soil to 10.85, 8.8, 6.10, 3.02 and 1.23 mg/g soil for 0, 1, 2, 3 and 4%, respectively. For pyrene, the result showed a decrease from 100 mg/g soil to 22.4, 14, 11.2, 6.55 and 3.24 mg/g soil for 0, 1, 2, 3 and 4%, respectively.

The positive influence of organic matter on the degradation process is a direct function of the ability of indigenous soil microbes to utilize the nutrients released in a plant available form. Accumulated organic matter serves as a storehouse of plant nutrients. This affirms that the more percent organic matter in the soil, the more nutrients and as such the higher the level of degradation. The Line weaver Burk modified Michaelis-Menten kinetics described by Levenspiel (1999) was used for the estimation of the specific reaction rate constant (k) for both naphthalene and pyrene. The specific rate constant approximates the fraction of the substrate present that is converted to product per small increment of time. The estimates are shown in Tables 6, 7, 8 and 9.

It was observed that there was an increase in the rate

Table 4. Effect of varying percent organic matter on naphthalene degradation level in soil.

Week	Bulk soil without organic matter	1% organic matter	2% organic matter	3% organic matter	4% organic matter
0	100	100	100	100	100
1	23.78	20.30	18.00	`5.50	25.10
2	70.65	17.01	16.01	13.20	23.20
3	18.40	15.00	13.00	10.25	16.55
4	15.20	12.02	10.11	7.55	12.23
5	10.85	8.8	6.10	3.02	1.23

Table 5. Effect of varying percent organic matter on pyrene degradation level in soil.

Week	Bulk soil without organic matter	1 % organic matter	2 % organic matter	3 % organic matter	4 % organic matter
0	100	100	100	100	100
1	33.07	30.55	28.50	25.10	20.2
2	32.00	28.12	26.20	23.20	15.12
3	30.15	25.80	23.00	16.55	9.33
4	25.80	20.20	18.10	12.23	4.25
5	22.40	14.00	11.20	6.55	3.24

constant with decreasing particle size fractions and increasing percent organic matter for both naphthalene and pyrene. Higher rates were observed when percent organic matter was varied when compared to variations in particle sizes. This phenomenon is again attributable to the positive influence of organic matter on biodegrade-tion. The correlation coefficients obtained using the linear regression method was quite high. This confirms the reliability of data obtained from experiments. Overall, clay soil fraction with 4% organic matter exhibited the best k value.

Table 6. Correlation coefficients and specific rate constants for naphthalene using different particle sizes.

Particle Size fraction	Correlation coefficient r^2	Reaction rate constant (k)
Coarse Sand	0.9045	0.125
Fine Sand	0.966	0.303
Silt	0.983	0.368
Clay	0.996	0.581

Conclusion

The mineralization of naphthalene and pyrene has been found to be a function of the particle size and varying percent of the organic matter content of the soil. This was due largely to the fact that large particle size fractions have little internal surface for PAH molecules to adhere

Table 7. Correlation coefficients and specific rate constants for pyrene using different particle sizes.

Particle size fraction	Correlation Coefficient r^2	Reaction rate constant (k)
Coarse sand	0.9045	0.125
Fine sand	0.966	0.303
Silt	0.983	0.368
Clay	0.996	0.581

Table 8. Correlation coefficients and specific rate constants for naphthalene with varying % organic matter.

% organic matter	Correlation coefficient	Specific rate constant (k)
0	0.734	0.441
1	0.815	0.556
2	0.827	0.625
3	0.942	0.743
4	0.987	0.95

and that finer particle size fractions, have more water, nutrients and soil organic matter retaining capacity. Since the soil moisture is an important compositional factor, the rate of biodegradation is thus increased as the mobility of these factors and the PAHs makes them available to themicrobes catalyzing the degradation. Biodegradation rate was found to increase in the order coarse sand $<$ fine sand $<$ silt $<$ clay and also in the following order for percent organic matter $0 < 1 < 2 < 3 < 4\%$. Aggregated

Table 9. Correlation coefficients and specific rate constants for pyrene with varying % organic matter.

% organic matter	Correlation coefficient	Specific rate constant (k)
0	0.731	0.221
1	0.880	0.409
2	0.897	0.493
3	0.932	0.574
4	0.976	0.768

soil (fine texture) has low C/N ratio, which implies high nitrogen content while large particles have high C/N ratios, which implies low nitrogen content.

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