AMM -17

Mineralization of Pyrene in Clay Soil Supplemented with Animal Organic Carbon Source

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Abstract
Biodegradation of polycyclic aromatic hydrocarbons (PAHs) is a major concern in the environment due to their toxic nature and ubiquitous occurrence. The study aimed to determine the best animal supplement of organic carbon source (cow dung, goat dung, pig dung and poultry (chicken) droppings) and indigenous microbes capable of mineralizing pyrene in clay soil. Clay soil sample was collected from the top surface soil (0 – 15cm) in Ikpoba River and upper Lawani river bank, both in Benin City with the aid of stainless steel trowel and gloved hands and stored in a polyethylene bag. The isolation and identification process was evaluated by staining method, microscopic examination and biochemical tests. The degradation experiment was conducted in bioreactors and supplemented cow dung, pig dung, goat dung and fowl droppings were added in varying amounts of 25 g, 50 g, 75 g and 100 g. The potential effects of animal organic carbon as nutrient supplements to enhance mineralization of pyrene were investigated. The rates of biodegradation of pyrene were studied for a remediation period of 35 days at the room of about 25°C. Results obtained from microbial isolation showed that *Bacillus substilis* and *Aspergillus niger* were the most populated in the soil and were therefore selected. The degradation experiment showed that mixed culture of *B. substilis* and *A. niger* using 50 g supplement with the dungs and droppings gave the highest percentage degradation with goat dung 97.8%, pig dung 98.1%, cow dung 97.7% and fowl droppings 98.3%. The least degradation was observed in supplement with 100g in a single culture of *A. niger* with fowl droppings yielded 64.5% degradation, pig dung 63.8%, goat dung 63.7% and cow dung 62.7%. The overall results of the study showed that 50g of animal wastes supplement in the ratio of 1:4 with pyrene contaminated soils are most effective in degradation processes.

Keywords: Biodegradation, microbes, pyrene, animal supplement, bioreactors

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds that originate from natural and anthropogenic pyrolysis of organic matter such as forest fire, automobile exhaust, coal refining processes and oil industry (Bouchez *et al.,* 1995). In 1980s, 16 polycyclic aromatic compounds were listed as priority pollutions by the US, Environmental Protection Agency, including pyrene (Kerstin *et al*., 2009).

Owabor *et al.* (2013) noted that the major environmental concern in urban and industrial area can be attributed to the increasing pollution particularly by these toxic chemicals such as pyrene, naphthalene, anthracene, benzo(a) pyrene, benzene, toluene, ethlbene and Xylene (BYTEX), heavy metals and dyes which are continuously discharged into the environment as an industrial waste, causing water, air and soil pollution. Pyrene is one of the group chemicals called PAH, which has four ring structures and it is colourless crystal-like solid but can also look yellow. Pyrene occurs in the various ecosystem and are priority pollutants due to their potential toxicity, mutagenic and carcinogenicity (Ali and Maram, 2010; Kalf *et al.,* 1997). The presence of pyrene is an environment has some effects on reproduction and mortality rates of aquatic animals, causes kidney diseases, causes tumor when inhaled or in contact with skin and increase in weight of liver in human (Chevron and Merlin, 2007). Also due to their hydrophobic nature, pyrene in aquatic and terrestrial ecosystem bind to particles in soil and sediments rendering them less available for plant uptake (Kerstin *et al.,* 2009). Microorganisms such as bacteria and fungi have been useful in effective degradation but it takes time and an attempt to achieve total clean-up with microorganisms alone, seem not successful. This led to supplementation of animal carbon source of carbon (Biostimulation) to speed-up the action of microorganisms in the remediation of soil contaminated by PAHs. The aim of this study was to determine the best animal supplement of organic carbon source for degradation of pyrene in clay soil.

Materials and Methods

The study was carried in the Department of Chemical Engineering Laboratory, University of Benin, Animal organic carbon source of Cow dung, Goat dung, Pig dung and Poultry (fowl) dropping was got from Federal University of Technology, Owerri, Farm

Limited. Sterilization of clay soil was carried out by autoclaving the soil sample at 120^{0}C for one hour, in order to remove the indigenous micro-organisms in the soil. The isolation and identification process was evaluated by staining method, microscopic examination and biochemical tests. Pyrene solution was prepared by dissolution of 250 mg of pyrene crystal in 1000 mls of distilled water with the aid of keem 80 surfactants, as surfactant increases the rate of PAH dissolution (Yeom et al., 1996) with the aid of magnetic stirrer for two hours in a conical flask. The final concentration was 250 mg/L pyrene. 200 g of sterilized soil was mixed with 100 ml distilled water in each of the bioreactors, labeled A to L respectively with a lid perforated to ensure adequate soil aeration. The experimental set-up was of four batches, with each batch consisting of twelve bioreactors. Batch one was labeled A_1 to L_1; consisting of cow dung. Batch two was labeled A_2 to L_2; consisting of goat dung. Batch three was labeled A_3 to L_3; consisting of pig dung. Batch four was labeled A_4 to L_4; consisting of poultry (chicken) dropping 80 ml of pyrene solution of 250 mg/l concentration was mixed in each of the bioreactors containing 200 g aqueous clay soil and was stirred very well and left for about 3days for the pyrene to adsorb in the soil sample. Bacteria and fungi degraders (*B. substilis* and *A. niger*) was introduced into the bioreactors to act as agent(s) of degradation. The soil solution (aqueous-soil) in each of the bio reactors was amended with different levels (25 g/l, 50 g/l, 75 g/l and 100 g/l) of organic carbon source of cow dung, goat dung, pig dung and poultry (chicken) dropping. The solution was transferred to the mechanical shaker for effective agitation. The organic carbon –amended soil was left to acclimatize. Sampling for quantitative analysis was carried out every seven days, by solid-liquid extraction method. Percentage degradation of pyrene is determined using below equation. $PC_i - PC_r) / PCi \times 100/1$, where PC_i depict the initial concentration of pyrene which is 250 mg/l. PC_r depicts residual concentration (g/kg) of pyrene obtained after each week (Adesodum and Mbagwu, 2008)

Results and Discussion

The results of the physiochemcial properties of the soil and animal dung amendments were depicted in table 1. The pH of the soil and animal dung amendments ranged from 6.5 to 7.1 with an average value of 6.8. Kastner and Malwo (1996) noted that pH very close to neutral or neutral improved the buffer capacities of the soil matrix as well as microbial activity. The results also revealed the high content of nutrients such as total nitrogen, available phosphorus and organic matter in the animal dung. This nutrient availability stimulates the growth of microorganism and biodegradation of the PAH-contaminated soils (Atlas and Bartha, 1998). Indigenous microbial degradation of pyrene in clay soil amended with animal organic carbon source supplements were shown in table 2. Different levels of amendment ranging from 25 g, 50 g, 75 g and 100 g supplement in the ratio of 1:8, 1:4, 1:2.7 and 1:2 of amendment agent to soil respectively. The result showed that the percentage degradation of pyrene among the levels of supplements decreases pattern of 50 g > 75 g > 25 > 100 g. There was a rapid reduction in the residual concentration of pyrene in all levels of supplements except 100g level supplement. This can be attributed to the reduction in the moisture content. Testa and Winegardner (1991) reported that when moisture content becomes lower than 10% of the water holding capacity, the bioactivity becomes marginal. It was noticed that degradation was rapid at the end of 21 days incubation period and subsequently become marginal during the rest of the incubation time. This occurs as a result of over inhibits aerobic biodegradation (Bossert and Bartha, 1984). Also, it was investigated by Ciccillo *et al.* (2002) that many microbial communities cease upon the addition of excess carbon sources.

Table 1: Physicochemical properties of soil and animal dung amendment

Parameter	Poultry dropping	Cow dung	Goat dung	Pig dung	Clay soil
pH	6.8	6.5	6.7	6.9	7.1
Total nitrogen (g/kg)	12.46	8.68	10.32	11.15	2.35
Phosphorus (mg/kg)	30.50	23.87	23.92	25.54	0.52
Organic carbon	42.58	31.15	31.24	34.03	10.89
Organic matter	73.41	53.70	53.86	58.67	18.77
Exchange acidity (cmol/kg)	0.27	0.26	0.25	0.30	0.06
H+ (cmol/kg)	0.16	0.14	0.13	0.18	0.02
Al^{3+} (cmol/kg)	0.11	0.10	0.12	0.12	0.04
Texture	NT	NT	NT	NT	Clay

NT= Not Tested

Fowl Dung: The degree of mineralization of pyrene in clay soil supplemented with fowl droppings showed a good reduction in the concentration of pyrene and fast degradation of pyrene (98.3%) and concentration (10.0 mg/l) was observed in 50 g mixed culture of *Bacillus substilis* and *A. niger*. *B. substilis* culture of 50 g had 95.3% of pyrene degradation and 11.6 mg/l concentration, whereas the

least degradation (62.7%) and concentration (92.9 mg/l) was found in 100 g supplement of *A. niger* culture.

Pig dung: 50 g supplement mixed culture of *B. substilis* and *Aspergillus niger* showed the highest percentage degradation (98.1) and concentration (4.7 mg/l), followed by 50 g of *B. substilis* culture with 97.4% pyrene degradation and 7.0 mg/l concentration. 100 g supplement of *A. niger* culture had the lowest degradation of 63.8% and concentration of 90.3 mg/l.

Goat dung: Mixed culture of *B. substillis* and *A. niger* with 50 g supplement gave the highest degradation of 97.8% and concentration of 5.4 mg/l. *B. substilis* culture with 50 g supplement showed 97.0% degradation and 7.2 mg/l concentration. The lowest degradation of 63.7% and 90.5 mg/l concentration was observed in *A. niger* culture with 100g supplements.

Cow dung: The mixed culture of *B. substilis* and *A. niger* with 50 g showed the highest mineralization of pyrene with 97.7% degradation and 5.6 mg/l residual concentration. 50g supplement in *B. substilis* culture has 97.0% degradation and 6.0 mg/l residual concentration. 100g supplement in *A. niger* had the least degradation of 64.3% and residual concentration of 88.6%. The highest rate of pyrene mineralization was achieved in the first 21 days of remediation. During this period, more than 50% of the pyrene concentration was mineralized, with a small and continual decrease in degradation until the end of remediation period (35 days). The percentage mineralization may be due to high nutrient level in the form of nitrogen and phosphorus provided by the added animal dung which stimulated an increase in intrinsic bacterial and fungi population and activity thus leading to high energy (carbon) demand by pyrene-degrading microbes. Similar observations have been reported for the use of pig dung (Agarry *et al.,* 2013; Yakubu, 2007), poultry dropping (Agarry, 2013) and goat dung (Agarry *et al.,* 2010) as biostimulating agent for petroleum hydrocarbon in soil.

Percentage degradation and concentration of pyrene after 35 day period of incubation with animal organic carbon source supplements

Animal waste				
Fowl Dropping				
Fowl droppings and *B. substilis*				
Level of amendment	100g	75g	50g	25g
Percentage degradation (%)	63.4	94.3	95.3	90.2
Concentration (mg/l)	91.3	14.0	11.6	24.2
Fowl droppings and *A. niger*				
Percentage degradation (%)	62.7	94.4	94.7	89.5
Concentration (mg/l)	92.9	14.0	13.0	26.0
Fowl droppings and *B. substilis* and *A. niger*				
Percentage degradation (%)	64.6	94.9	98.3	76.5
Concentration (mg/l)	88.4	12.6	10.0	58.5
Pig Dung:				
Pig dung and *B. substilis*				
Level of amendment	100g	75g	50g	25g
Percentage degradation (%)	64.7	96.5	97.4	90.3
Concentration (mg/l)	88.0	8.6	7.0	24.04
Pig dung and *A. niger*				
Percentage degradation (%)	63.8	95.6	96.5	89.3
Concentration (mg/l)	90.3	10.8	8.6	26.6
Pig dung and *B. substilis* and *A. niger*				
Percentage degradation (%)	66.0	97.5	98.1	90.9
Concentration (mg/l)	84.8	6.0	4.7	22.7
Goat Dung:				
Goat dung and *B. substilis*				
Level of amendment	100g	75g	50g	25g
Percentage degradation (%)	64.7	96.8	97.0	89.8
Concentration (mg/l)	88.2	7.8	7.2	25.4
Goat dung and *A. niger*				

Percentage degradation (%)	63.7	96.1	96.7	89.0
Concentration (mg/l)	90.5	9.6	8.6	27.4
Goat dung and *B. substilis* and *A. niger*				
Percentage degradation (%)	65.9	97.6	97.8	90.7
Concentration (mg/l)	85.0	6.0	5.4	23.1
Cow Dung:				
Cow dung and *B. substilis*				
Level of amendment	100g	75g	50g	25g
Percentage degradation (%)	65.0	96.1	97.0	96.6
Concentration (mg/l)	87.4	9.6	6.0	8.2
Cow dung and *A. niger*				
Percentage degradation (%)	64.5	95.5	96.3	95.4
Concentration (mg/l)	88.6	11.0	9.2	11.4
Cow dung and *B. substilis* and *A. niger*				
Percentage degradation (%)	65.8	96.8	97.7	97.5
Concentration (mg/l)	85.4	7.8	5.6	6.0

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