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Bioremediation of Total Polycyclic Aromatic Hydrocarbon Contaminated Soil Using Nitrified Sawdust and *Pseudomonas auriginosa*

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Abstract

Bioremediation involving bioaugmentation and biostimulation are eco-friendly existing methods for degrading polycyclic aromatic hydrocarbons (PAHs) in contaminated soils. This study investigates the efficiency of *Pseudomonas auriginosa* and nutrient-enriched sawdust (SD) in biodegrading ∑PAHs in contaminated soil (CS). Four compost mixtures of CS/SD (1:0, 3:1, 1:1, 1:3) were applied for 2, 4, 6, 8-week bio-cleanup after inoculation. Results show ∑PAHs concentrations decreased with increasing time of treatment for all four compost in experimental and control setups. The removal efficiency of ∑PAHs was clearly associated with nutrient-enriched sawdust and *Pseudomonas auriginosa,* especially for 3:1, 1:1, and 1:3 ratios. Both factors had a significant effect ($p = 0.05$) on removal efficiency compared to the control setup. The highest (78.5%) and lowest (37.8%) ∑PAHs removal efficiency were observed for CS/SD ratios of 1:3 and 1:0 respectively after 8-week treatment. In this instance, this study recommends a CS/SD ratio of 1:3 at 8-week treatment to achieve maximum removal efficiency of ∑PAHs in contaminated soils.

Keywords

Bioremediation, Sawdust, Composting, Contaminated Soil, Polycyclic Aromatic Hydrocarbon

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are of wide, regional and international concern due to their teratogenicity, carcinogenicity and ecotoxicity. They are one of the most prevalent hazardous contaminants sink in soils arising primarily from anthropogenic and natural sources [1]. The typology of these contaminants is structurally based-linear, angular, or cluster [2] [3] [4] [5] with 2 - 3 benzene

rings with Low Molecular Weight (LMW) and over 3 benzene rings with High Molecular Weight (HMW). The HMW polycyclic aromatic hydrocarbons are considered highly toxic and microbial recalcitrant [6] [7] and therefore classified as priority pollutants with the highest human carcinogens by the U.S. EPA and European Union [8].

Globally, about 90% of the environmental PAHs burden is present in soils [9]. Indeed, the increasing rate of soil contamination with PAHs constitutes environmental sustainability challenge. Remediation is therefore of great importance to subvert the effects of these contaminants. This has led to the application of various remediation methods in order to achieve environmental sustainability in line with the global Sustainable Development Goals (SDGs) framework. Some future and emerging remediation technologies are in the development stages and current existing methods like physical methods [10], chemical methods [11] [12] [13] and biological processes, where soil microbial activities are enhanced for effective biodegradation of PAHs compounds [14] [15] [16]. Among these methods, bioremediation involving the application of microorganisms either through bioaugmentation, biostimulation, composting and the likes has been proven extremely viable and cost-effective [14] [16] [17].

This study applies laboratory-scale bioreactors to investigate the efficiency of *Pseudomonas auriginosa* with nutrient-enriched sawdust composting in bioaugmenting, biostimulating degradation of polycyclic aromatic hydrocarbon contaminated soil. Furthermore, a variable such as treatment time and the ratio of contaminated soil to sawdust were examined for optimal bio-cleanup and percentage removal efficiency of PAHs-contaminated soil.

2. Materials and Methods

2.1. Contaminated Soil and Sawdust Sampling

Polycyclic aromatic hydrocarbon contaminated soil (about 10 kg) was collected from oil polluted site and sawdust (uncontaminated with PAH - 10 kg) was collected from remote locations free from oil pollution using clean sterilized hand trowel. The samples were transported to laboratory in black polyethylene wrap aluminium foil bags. Both contaminated soil and sawdust were air dried at room temperature, homogenized and sieved through 4mm mesh size sieve and stored for laboratory scale bioremediation experiment and analysis.

2.2. Rector Design

The bioreactor design was adopted from [14] with slight modifications. Eight 5 L capacity (fitted with stainless steel starrier) polyvinylchloride aerated vessels were arranged in sequence for laboratory-scale PAHs-bioremediation experiment. The bioreactor vessels where connected with warm air delivery pump (1.8 kw 8 bar 25 L tank capacity-195 L/Min) via glass tubing's. Gaseous air streams from inlet and exhaust were linked to sodium hydroxide reservoir to monitor carbon dioxide emission which is evidence of biodegradation. The set-up was

operated continuously for 8 weeks, which have previously been proven to achieve maximum PAHs removal efficiency [18] [19].

2.3. Experimental Design

Each bioreactor vessels had a holding capacity of 1000 g of total composting mixture (contaminated soil-to-sawdust). The experimental design involved composting contaminated soil (CS) and sawdust (SD) in following ratios; 1:0, 3:1, 1:1 and 1:3. For the bioremediation experiment, nutrient-enriched sawdust was composted with contaminated soil which seeks to investigate its potential enhancement of removal efficient of PAHs in soil. The compost ratios were inoculated with 100 mL *Pseudomonas auriginosa* to evaluate effect of microbes on biodegradation of PAHs contaminated soils. Control compost consisted same (CS:SD) ratios but without nutrient-enriched sawdust (*i.e.*, only sawdust) and microbial inoculation. The experiment was setup for 8 continuous weeks at mesophilic temperature (30˚C ± 2˚C) by aerating each reactor with warm atmospheric air with constant daily stirring. Humidity of reactors maintained at 50% moisture content using sterilized distilled water during the experiment. Samples from each reactor were collected in duplicate after 0, 2, 4, 6, and 8 weeks, homogenised and stored in amber glass bottle for PAHs analysis.

2.4. Chemical Analysis

Sawdust were enriched with supplementary substrate solutions of nitrate, phosphate, potassium (99.8% Sigma-Aldrich, St. Louis, MO, USA) and homogenised. Before bioremediation experiment, the contaminated soil and compost were subjected to initial characterization. pH were measured in deionized water using a solid:liquid ratio of 1:1 (w/v) following 1 hour shaking and filtration through a Whatman (Cat No 1001, 110 mm) filter paper using pH meter (Hanna Instrument). Particle size analysis was carried out using the hydrometeric method [20]. Soil organic carbon content by Walkley and Black method [21], available phosphate was determined by the Bray No. 1 method [22], soil nitrate was determination by distillation method following extraction with 2M KCL solution [23]. Potassium by atomic emission spectrophotometry after digesting with 6M HNO_3.

2.5. PAHs Extraction, Cleanup and Analysis

PAHs were extracted from contaminated soil and composting mixtures through solvent extraction system with a 1:1 (v/v) acetone/dichloromethane mixture. Samples were extracted at 100˚C. Extracts pooled, dried over anhydrous sodium sulphate, concentrated with a rotary evaporator and solvent exchanged with 5 mL of n-hexane. Sample cleanup procedure was done on a silica gel-aluminum oxide column (10 cm × 6 mm ID). Elution was carried out by successive loading 5 mL n-hexane and 20 mL n-hexane-dichloromethane (3:7, v/v) and second part of the elution collected, sealed and kept for PAHs analysis. PAHs quantification was conducted using Agilent 7890 - 5975 c gas chromatography-mass spectro-

photometer (GC-MS). A DB-5 column (30 cm × 0.25 mm × 0.10 μm) was used. The injection volume was 1μL. Helium was used as carrier gas at a flow rate of 1 mL/min. Column temperature was set at 50˚C for first 1 min, increased 20˚C/min to a temperature of 120˚C, then increased 4˚C/min - 310˚C, and maintained at 310˚C for 30 min. Mass spectrophotometer condition were: electron impact, electron energy 70 eV; filament current 100 μA; multiplier voltage, 1200 V; full scan. Quantification of individual PAHs was performed in MS/MS scan mode at normal speed, based both on retention time and characteristic ions. Concentrations of each PAHs were finally calculated and calibrated using standard calibration graph [24] [25].

2.6. Statistical Quality Control and Assurance

Statistical analysis of results was done using Paleontological Statistic software (PAST version 1.38) and Microsoft Excel (2012 version). Average results were reported on wet weight bases. Cluster analysis was used to establish distinct grouping of compost and experimental time for efficient ∑PAHs removal while comparison of removal efficiency of composted mixtures and control were perform using t-testing and analysis of variance. Split extracts were incorporated for instrument data validation with t-test showing no significant differences ($p < 0.05$) in actual and split results. Analar grade (Sigma-Aldrich) reagents were used for the experiment.

3. Results and Discussion

3.1. Characterization of Contaminated Soil and Sawdust

Table 1 shows initial characterization of contaminated soil and nitrified sawdust at different experimental ratios. Average pH values of four CS/SD ratios were within neutral optimum range of 7.33 - 7.84 with sandy loam texture characteristic of tropical humid environment. Nutrient levels of each CS/SD ratios (organic carbon, nitrate, phosphate, potassium) showed progressive increase, *i.e.* 1:0 < 3:1 < 1:1 < 1:3 has factored in the experimental design. Total PAHs concentration was highest in 1:0 - 817 ± 7.83 μg/g due to absence of sawdust composting and decreases exponentially in relation to sawdust ratio to 3:1 - 520 ± 4.97 μg/g; 1:1 - 464 ± 5.65 μg/g; 1:3 - 459 ± 4.33 μg/g. The PAHs degrader was maintained at 2.0×10^3 CFU/g for all ratios. PAHs contaminated tropical soils have advantage over arid soils for easy degradation due to its characteristic.

3.2. Percentage Removal Efficiency of PAHs

Bioremediation techniques are extremely successful at remediating polycyclic aromatic hydrocarbon contaminated soils since it's an in-situ process. In addition, it appears to be more environmentally acceptable than other techniques. Laboratory scale bioreactors were used to investigate the feasibility of applying *Pseudomonas auriginosa* with nutrient-enriched sawdust composting in bioaugmenting, biostimulating degradation of PAHs contaminated soil. Total PAHs

Table 1. Initial characterization of contaminated soil and compost mixtures.

Parameter	CS/SD Ratios			
	1:0	3:1	1:1	1:3
pH	7.62 ± 0.23	7.74 ± 0.25	7.84 ± 0.13	7.33 ± 0.29
% Organic carbon	8.36 ± 2.10	18.8 ± 1.18	21.1 ± 1.18	23.5 ± 1.26
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Nitrate (mg/kg)	84.3 ± 8.36	217 ± 6.84	233 ± 5.85	314 ± 5.88
Phosphate (mg/kg)	6.13 ± 0.94	17.3 ± 0.73	19.6 ± 1.10	24.9 ± 1.18
Potassium (mg/kg)	12.4 ± 2.10	189 ± 1.43	222 ± 1.25	310 ± 1.64
Hydrocarbon utilizing bacteria count (CFU/g)	2.0×10^3	2.0×10^3	2.0×10^3	2.0×10^3
Total PAHs (∑μg/g)	817 ± 7.83	520 ± 4.97	464 ± 5.65	459 ± 4.33

CS: Contaminated soil. SD: Sawdust.

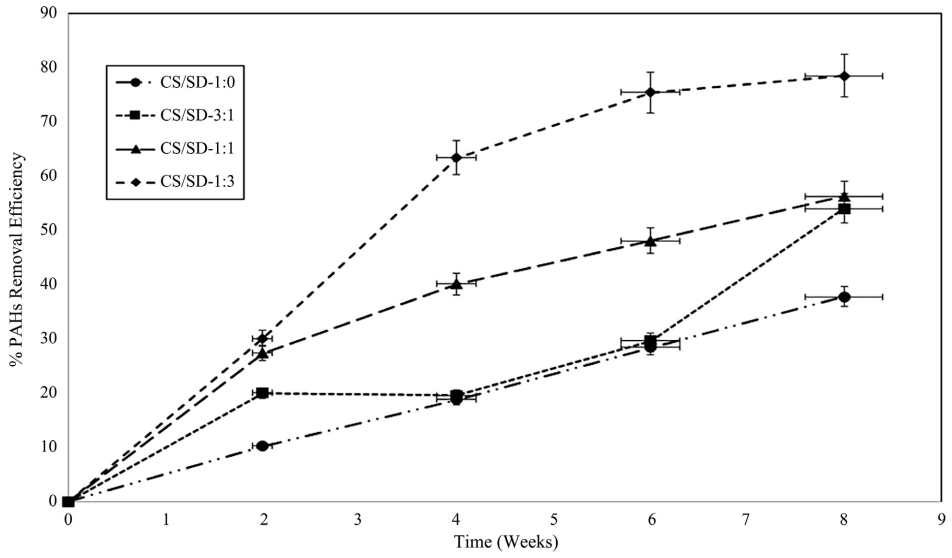
concentrations in experimental (with *Pseudomonas auriginosa and nitrified sawdust*) and control (without *Pseudomonas auriginosa and nitrified sawdust*) setups were generally observed to decrease with time for all four CS/SD ratios (Table 2). Decreasing rate was more pronouns in experimental than control setup. Total polycyclic aromatic hydrocarbon concentration (817 ± 7.83 μg/g) in CS:SD ratio 1:0 was higher due to absence of sawdust. For experimental setup after 8 weeks; observed remediation factors were CS/SD 1:0 - *1.6*; CS/SD 3:1 - *2.2*; CS/SD 1:1 - *2.3*; CS/SD 1:3 - *4.7*. Furthermore, analysis of variance ($p = 0.05$) showed significant difference in ∑PAHs concentrations within the four CS/SD ratios. These factors show improved bioremediation of the PAHs contaminated soil. The increase in ∑PAHs degradation in experimental setup especially for ratios 3:1, 1:1 and 1:3 with respect to time can be attributed to the effect of nutrient-enriched sawdust and *Pseudomonas auriginosa* inoculation. The higher the organic carbon content, nitrate, phosphate and potassium levels the higher the degradation efficiency (Table 2). Comparatively, control had much higher ∑PAHs concentrations to experimental setup with respect to time and composting ratio, with corresponding low remediation factors of CS/SD 1:0 - *1.2*; CS/SD 3:1 - *1.2*; CS/SD 1:1 - *1.2* and CS/SD 1:3 - *1.4*. Sawdust enhances sorption of PAHs thereby reducing their bioavailability. The more enriched sawdust to contaminated soil, the greater the bioremediation process [18]. Bioaugmentation with pre-grown microbial inoculation and biostimulation with nutrient enrich media have clear advantage in enhancing bioremediation of PAHs contaminated soils [26]. Several strains of bacteria have been reported to have a high PAHs biodegradation potential [27] [28]. However, their potentials greatly depend on several factors like, temperature, pH, aeration, organic matter, nutrient levels, moisture content and soil type [19] [29] [30].

Figure 1 shows percentage removal efficiency of ∑PAHs from experimental and control setup. A progressive increase was observed in removal efficiency of ∑PAHs with time. However, removal efficiency was significantly higher ($p = 0.05$)

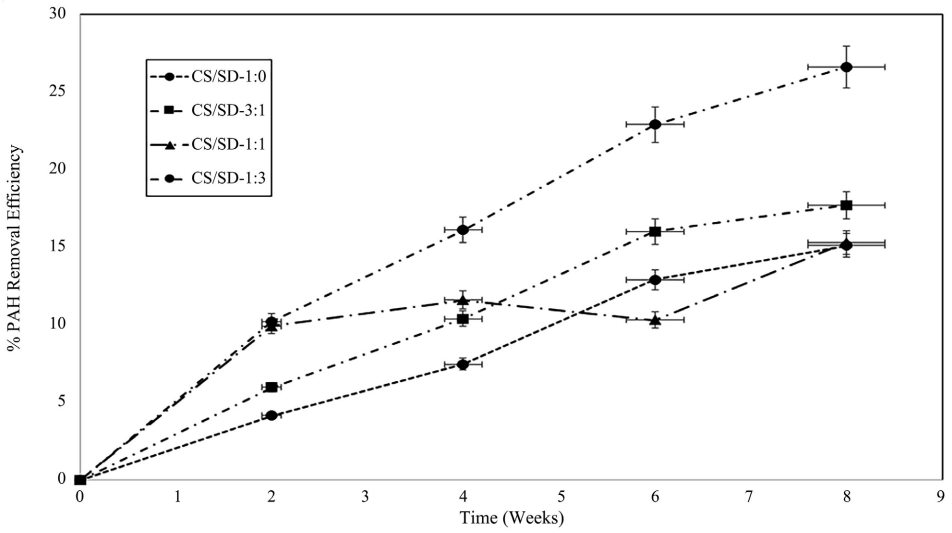
Table 2. Total PAHs concentrations and % removal efficiency.

CS/SD Ratio	Weeks	Experimental		Control	
		Total PAHs (µg/g)	% Removal Efficiency	Total PAHs (µg/g)	% Removal Efficiency
1:0	0	817 ± 7.83	0	817 ± 3.26	0
	2	734 ± 3.89	10.2	783 ± 3.03	4.16
	4	663 ± 3.77	18.8	756 ± 2.18	7.47
	6	585 ± 4.36	28.4	712 ± 2.11	12.9
	8	508 ± 3.21	37.8	694 ± 2.90	15.1
3:1	0	520 ± 4.97	0	520 ± 2.64	0
	2	463 ± 2.15	20.0	489 ± 2.18	5.96
	4	418 ± 2.84	19.6	466 ± 1.94	10.4
	6	366 ± 3.04	29.6	437 ± 2.55	16.0
	8	239 ± 2.23	54.0	428 ± 2.42	17.7
1:1	0	464 ± 5.65	0	464 ± 3.23	0
	2	337 ± 2.17	27.4	418 ± 2.84	9.91
	4	278 ± 2.93	40.1	410 ± 1.66	11.6
	6	241 ± 2.11	48.1	411 ± 2.04	10.3
	8	203 ± 3.10	56.3	393 ± 1.21	15.3
1:3	0	459 ± 4.33	0	459 ± 2.85	0
	2	321 ± 1.95	30.1	412 ± 2.77	10.2
	4	168 ± 2.18	63.4	385 ± 2.10	16.1
	6	113 ± 2.04	75.4	354 ± 1.09	22.9
	8	98.6 ± 3.16	78.5	337 ± 2.24	26.6

% Removal efficiency = ∑PAHs at 0 week-∑PAHs at 2, 4, 6, 8 weeks/∑PAHs × 100.



(a)



(b)

Figure 1. % removal efficiency of ∑PAHs from experimental (a) and control setup (b).

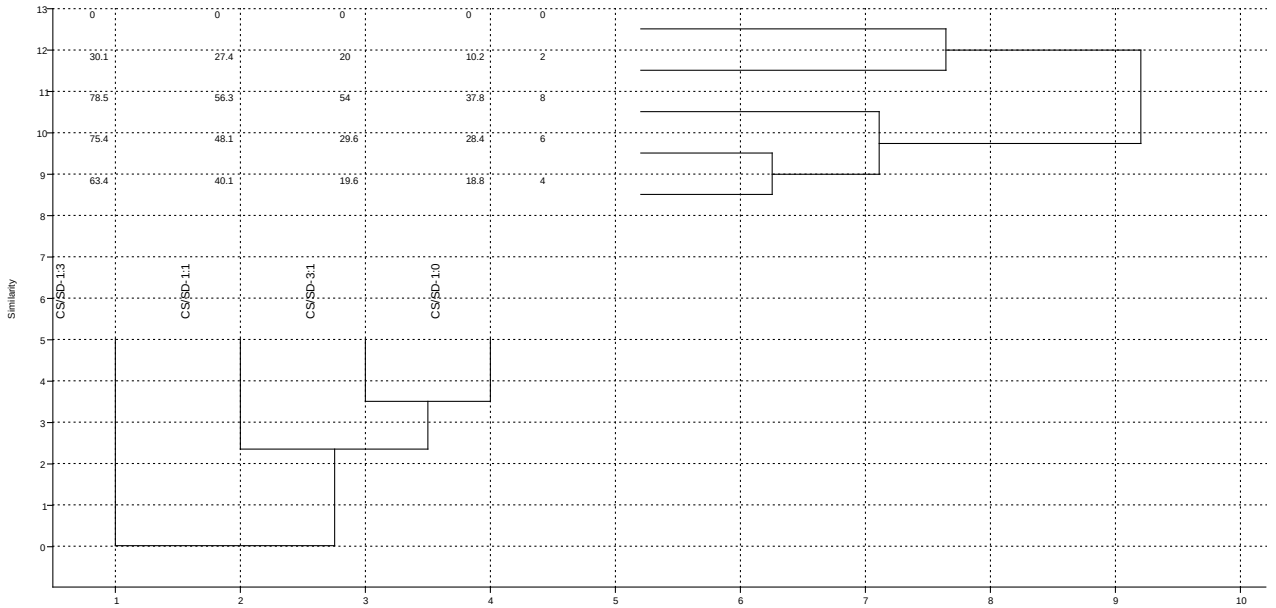


Figure 2. Euclidean paired group cluster analysis of inoculated ∑PAHs in contaminated soil.

for experimental setup; CS/SD 1:0 - 37.8%; CS/SD 3:1 - *54.0%*; CS/SD 1:1 - *56.3%* and CS/SD 1:3 - *78.5%* than control at 8 weeks (Table 2). Statistical t-testing (*95% confidence limit*) showed significant difference in removal efficiency of ∑PAHs from experimental composted soil to control. Hydrocarbon utilizing bacteria—*Pseudomonas auriginosa* with nutrient-enriched sawdust effectively aided in bioremediation of the PAHs contaminated soil as evident in the t-testing. Euclidean paired group cluster analysis of inoculated PAHs composted soils shows three distinct groups associated with composting ratios and time of similar data sets (Figure 2). For compositing; CS/SD ratios 1:0 & 3:1 had about the same low removal efficiency, while 1:1 and 1:3 reported higher efficiencies.

These clusters also clearly show the advantage of nutrient-enriched sawdust in the bioremediation process. For time variable; 0/2, 8 and 4/6 weeks were observed. This multivariate analysis confirms compost of 1:1, 1:3 and 4, 6 and 8 weeks to be most efficient for ∑PAHs removal from contaminated soils.

4. Conclusion

The laboratory-scale experimental findings showed that under the right bioremediation conditions, hydrocarbon utilizing bacteria—*Pseudomonas auriginosa* in conjunction with nutrient-enriched sawdust was capable of degrading PAHs contaminated soil. This is evidently demonstrated by the compost ratios of 3:1, 1:1 and 1:3. Removal efficiency improved remarkably with longer bioremediation time. After 8 weeks 54.0%, 56.3% and 78.5% removal efficiency was achieved for 3:1, 1:1 and 1:3 respectively. The optimal removal efficiency was recorded for 1:3 at 8 weeks. Further, it is necessary to investigate the correlation relationship between different nutrient levels with corresponding PAHs removal efficiency and possibly examine other types of bacteria for maximum efficiency.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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