



Effect of bacterial co-culture and organic amendments on the bioremediation of hydrocarbons in a soil contaminated with spent engine oil

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ABSTRACT. Bioenhancement of hydrocarbonoclastic microorganisms with suitable nutrients has a huge impact in achieving positive bioremediation of polluted environments. This study was conducted to assess the bio-enhancing effect of some organic amendments on *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture with a view to remediating spent engine oil (SEO) contaminated soil. Top soil (1.5 kg) was autoclaved and thereafter contaminated with SEO at three levels. The contaminated soil was inoculated with bacterial co-culture (150 mL) and subsequently bioenhanced with compost, processed cocoa pod husk (CPH) and cow dung. The factorial experiment was laid out in completely randomized design. Concentrations of total petroleum hydrocarbon (TPH) and selected polycyclic aromatic hydrocarbons (PAH) were estimated on the first day, 5th week and 10th week of incubation. Results obtained show that bacterial co-culture bioenhanced with compost produced the most significant TPH reductions (1318 and 261 mg kg⁻¹) on 10% SEO contaminated soil at the 5th and 10th week respectively ($p < 0.05$). Again, bacterial co-culture bioenhanced with compost produced the most significant PAH reductions (65.9 and 55.8 mg kg⁻¹) on 10% SEO contaminated soil at the 5th and 10th week respectively ($p < 0.05$). The significant bioremediation capabilities exhibited by the bacterial co-culture bioenhanced with organic amendments in this study has made these bioremediation agents potential candidates in remediating soils impacted with petroleum hydrocarbons.

Keywords: bioenhancement; spent engine oil; organic amendments; bacterial co-culture; soil.

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Introduction

Bioremediation which is the employment of living organisms, most particularly microorganisms to degrade pollutants in the environment into less hazardous forms, has produced auspicious results coupled with the fact that it is economically and environmentally friendly (Nkereuwem et al., 2020). However, the aptitude of these hydrocarbonoclastic microorganisms to utilize hydrocarbons has been credited by Ijah, Safiyanu, and Abioye (2008) to their enzymatic prowess and capability to subsist punitive environmental conditions. According to Bundy, Paton, and Campbell (2002); Atagana (2008); Al-Sulaimani, Al-Wahaibi, and Al-Bahry (2010), biodegradation of hydrocarbons in the soil can be restricted by numerous factors; nutrients, presence of pollutants, pH, temperature, moisture, oxygen and inherent soil properties.

The total estimation of total petroleum hydrocarbon (TPH) in environmental media has enabled the application of proper bioremediation techniques that could be applied in the rejuvenation of the contaminated sites (Schwartz, Ben-Dor, & Eshel, 2012). These authors further reiterated that it is vital to study the effects of hydrocarbon type and soil properties on the extraction efficiency coupled with its repeatability across laboratories. It has been established by Yerima et al. (2013); Umana, Uko, Bassey, and Essien (2017), that if spent engine oil (SEO) pollutes agricultural soil, the presence of polycyclic aromatic hydrocarbons (PAH) that it possesses would add to long-term (chronic) hazards including carcinogenicity, toxicity, mutagenicity and teratogenicity.

The utmost need to remediate hydrocarbon environments is linked to the ill effects it portends coupled with its ability to render agricultural land unproductive. According to Onwurah, Ogugua, Onyike, Ochonogor, and Otitoju (2007), petroleum hydrocarbons have got the capability to sterilize agricultural soil thereby stopping crop growth and yield. The adoption of bioaugmentation and biostimulation as appropriate technologies in remediating hydrocarbon impacted soil has yielded positive results over the years. The vital advantage of biostimulation is that it will be carried out by already present indigenous microbial community that is compatible with the subsurface environment, and well dispersed spatially within the subsurface (Adams, Fufeyin, Okoro, & Ehinomen, 2015). It is based on this background information that this study was conducted with a view to assessing the possibility of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture that was bioenhanced with organic amendments in expunging the hydrocarbon contents of SEO contaminated soil.

Material and methods

Description of the study area

This study was conducted at the Department of Soil Science, Faculty of Agriculture, Federal University Dutse campus Jigawa state, Nigeria which is located at Latitude 11° 46' 39" North and Longitude 9° 20' 3" East. Onokebhagbe et al. (2021) reported that the study area is categorized as a typical Sudan savanna agro-ecological zone.

Collection and processing of organic amendments

Organic amendments; cow dung, cocoa pod husk (CPH) and compost that was generated from composting of fresh CPH and cow dung were all collected and processed according to the procedures already reported by Adeleye et al. (2020).

Collection of soil and spent engine oil

With the aid of an auger, top soil (250 kg) that had no history of pollution was collected at the depth of 25 cm from four (4) different points at the back of the Department of Soil Science, Federal University Dutse, Nigeria. As conducted by Soretire, Oshiobugie, Thanni, Balogun, and Ewetola (2017), the soil was air dried, bulked and subsequently sieved with two (2) millimeter mesh size. Eight (8) liters of SEO was collected from a service pit in Dutse mechanic village, Jigawa state, Nigeria.

Isolation and identification procedures for bacterial inoculants

Spent engine oil utilizing bacteria; *S. pyogenes* and *E. faecalis* whose co-culture was employed for the bioaugmentation of the SEO contaminated soil in this study were isolated and identified as earlier reported by Adeleye et al. (2022).

Preparation of SEO contaminated soil

Sieved soil was autoclaved following the procedure earlier reported by Adeleye et al. (2020). Following the procedure of Agbor et al. (2015), one thousand and five hundred grammes (1500 g) of the autoclaved soil was put in thirty six (36) polyethylene bags and 5, 10 and 15% SEO were added intentionally. The soil and the added SEO were then cautiously mixed and made to stand undisturbed under room temperature for fourteen (14) days with a view to enabling the toxic components volatilize as reported by Abioye, Agamuthu, and Abdul-Aziz (2012).

Bioremediation experiment

The factorial experiment (4 x 3) was laid out in a completely randomized design with three replicates for each SEO contamination level whereby thirty six (36) polyethylene bags were set up. As reported by Ezekoye, Amakoromo, and Ibiene (2017), out of the thirty six (36) polyethylene bags, nine (9) polyethylene bags were kept as control while one hundred and fifty grammes (150 g) of each organic amendment; compost, CPH and cow dung was added and expansively mixed with the soil contaminated with 5, 10 and 15% SEO. Excluding the nine (9) polyethylene bags used as control, all the remaining twenty seven (27) polyethylene bags were

comprehensively mixed with one hundred and fifty milliliters (150 mL) of the bacterial co-culture. All the polyethylene bags were subjected to room temperature incubation for seventy (70) days as reported by Chorom, Sharifi, and Motamedi (2010). As reported by Chorom et al. (2010), the contents of the polyethylene bags were tilled twice in a week to ensure good aeration while the moisture content was kept constant by adding six milliliters (6 mL) distilled water two times in a week as reported by Abioye et al. (2012). Estimations of TPH and PAH in the SEO contaminated soil were done on the first day, fifth week and tenth week that the experiment lasted.

Estimations of total petroleum hydrocarbon and polycyclic aromatic hydrocarbons

Total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAH) in the SEO contaminated soil were estimated through the adoption of gas chromatograph flame ionization detector (GC-FID) system following the procedures documented by United State Environmental Protection Agency [Usepa] (2003).

Determination of physicochemical parameters of soil and organic amendments

Physicochemical properties of the soil and organic amendments ranging from pH and electrical conductivity (EC), organic carbon, total nitrogen, potassium, cation exchange capacity (CEC), phosphorous and other relevant parameters were determined following the procedures earlier reported by Adeleye et al. (2020).

Data analysis

Data collected were subjected to procedure of general linear model of GenStat version 17 and significant means were subsequently separated through Duncan's Multiple Range Test (DMRT).

Results and discussion

The CEC of the soil and compost used in this study recorded 3.51 cmol kg⁻¹ and 221.7 cmol kg⁻¹ respectively. Compost recorded the most (48.25 %) organic carbon as well as the most (5.85 %) total nitrogen (Table 1).

Table 1. Physicochemical properties of soil and organic amendments

Properties	Soil	CPH	Compost	Cow dung
Moisture content (%)	2.04	11.11	2.0	7.3
Ash content (%)	-	23	65	68.8
pH _(water)	6.5	7.6	9.45	8.15
Organic Carbon (%)	0.49	33.40	48.25	41.55
Total Nitrogen (%)	0.06	2.65	5.85	2.85
Available Phosphorous (mg kg ⁻¹)	11.02	0.08	1.48	1.2
EC (dS cm ⁻¹)	0.92	6.42	8.86	8.10
Exchangeable Bases (cmol kg ⁻¹)				
Potassium	0.19	162	213.16	80
Calcium	1.82	1.6	4.8	0.2
Magnesium	0.92	2.45	3.24	1.5
Sodium	0.58	0.1	0.5	0.4
CEC	3.51	166.15	221.7	82.1
Particle Size (g kg ⁻¹)				
Clay + Silt	420	-	-	-
Clay	100	-	-	-
Silt	320	-	-	-
Sand	580	-	-	-
Textural class	Sandy Loam	-	-	-

Note: - = No value; CPH= Cocoa pod husk

Baseline concentrations of total petroleum hydrocarbon and polycyclic aromatic hydrocarbons in the SEO contaminated soil

The results generated from the estimation of TPH and PAHs using GC FID on the first day of SEO contamination of the experimental soil are depicted in Table 2. In terms of TPH concentrations in the varying SEO contaminated soil, experimental bag with 5% SEO contamination recorded the least TPH concentration (9934 mg kg⁻¹) while the one with 15% SEO contamination recorded the most TPH concentration (10379 mg kg⁻¹).

Table 2. Concentrations of hydrocarbons in spent engine oil contaminated soil before bioremediation.

	Concentration of spent engine oil in contaminated soil (mg kg ⁻¹)		
	5%	10%	15%
TPH (Day one)	9934	10016	10379
PAH (Day one)	1065.9733	1726.5850	1865.6340

Note: TPH= Total petroleum hydrocarbons; PAH= Polycyclic aromatic hydrocarbons

Total petroleum hydrocarbon biodegradation potential of *S. pyogenes* and *E. faecalis* co-culture

All the organic amendments significantly enhanced TPH degradation facilitated by *S. pyogenes* and *E. faecalis* co-culture in this study ($p < 0.05$). Remarkably, at the fifth week, compared to other organic amendments, powdered CPH, compost and powdered cow dung enhanced the most TPH reductions (3687 mg kg⁻¹, 1318 mg kg⁻¹ and 3821 mg kg⁻¹) compared to other organic amendments on 5%, 10% and 15% SEO contamination levels respectively (Table 3). At the tenth week, further TPH reductions were recorded as compost enhanced the most TPH reductions (328 mg kg⁻¹ and 261 mg kg⁻¹) compared with others on 5% and 10% SEO contamination levels respectively (Table 4).

Table 3. Effect of organic amendments on bacterial total petroleum hydrocarbon degradation (mg kg⁻¹) at the fifth week.

Organic amendments	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	8558 ^f	1318 ^l	5846 ^g
CPH	3687 ^k	8801 ^e	9806 ^d
Cow dung	4027 ^h	3753 ^j	3821 ⁱ
Control	9934 ^c	10017 ^b	10379 ^a

Note: Means with the same letters in each column are not significantly different using Duncan's multiple range test (DMRT) ($p > 0.05$).

Table 4. Effect of organic amendments on bacterial total petroleum hydrocarbon degradation (mg kg⁻¹) at the tenth week.

Organic amendments	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	328 ^k	261 ^l	1264 ^h
CPH	1403 ^g	1677 ^f	2570 ^d
Cow dung	1760 ^e	866 ^j	1007 ⁱ
Control	9934 ^c	10017 ^b	10379 ^a

Note: Means with the same letters in each column are not significantly different using Duncan's multiple range test (DMRT) ($p > 0.05$).

However, powdered cow dung enhanced the most TPH reduction (1007 mg kg⁻¹) compared with other organic amendments on 15% SEO contamination level (Table 4). These results are in agreement with the reports of Boontawan & Boontawan (2011); Kumar, Dhanarani, and Thamaraiselvi (2013); Wanjohi, Mwamburi, Too, Aloo, and Kosgei (2015) on the ability of *S. pyogenes* and *E. faecalis* to degrade hydrocarbons. The TPH reductions recorded in this study might be due to the ability of *Alcaligenes faecalis* to produce biosurfactants which aid efficient bioavailability of hydrocarbons for effective degradation. This claim has been reported by Igwo-Ezikpe, Gbenle, Ilori, Okpuzor, and Osuntoki (2009); Bharali, Das, Konwar, and Thakur, (2011); Wokem, Odokuma, & Ariole (2017).

The supplementation of the hydrocarbonoclastic bacterial co-culture employed in this study with organic amendments has proved to be very effective in achieving desirable bioremediation of SEO. The feat that the bacterial co-culture attained in terms of reducing the concentrations of TPH from the SEO contaminated soil studied can be credited to its active supplementation with apposite nutrients that eventually enhanced its performance. Similar results achieved in this study have been reported by Garcia-Gomez, Roig, and Bernal (2003); Okolo, Amadi, and Odu (2005); Manios et al. (2006); Marin, Moremo, Hernandez, and Garcia (2006); Ibekwe, Ubochi, and Ezeji (2006); Awodun (2008); Gopamma and Srinivas (2011); Orji, Abiye, and Dike (2012). The capability of organic amendments to enhance effective remediation of SEO polluted soil owing to its improved soil chemical properties and microflora has been reported by Nwogu and Azubuike (2015).

Biodegradation experiment of polycyclic aromatic hydrocarbons by a co-culture of *S. pyogenes* and *E. faecalis*

Findings recorded on the potential of *S. pyogenes* and *E. faecalis* co-culture to degrade the PAH contents of SEO contamination levels employed in this study revealed that the organic amendments employed significantly enhanced such ($p < 0.05$). Specifically, at the fifth week, powdered CPH recorded the most PAHs

reduction (499.9 mg kg^{-1}) compared with other organic amendments on 5% SEO contamination level while compost enhanced the most PAH reductions (65.99 and $585.99 \text{ mg kg}^{-1}$) compared with other organic amendments on 10% and 15% SEO contamination levels respectively (Table 5). Fascinatingly, at the tenth week, compost continued this trend by enhancing the most PAH reductions (111.69 and 55.89 mg kg^{-1}) compared with other organic amendments on 5 and 10% SEO contamination levels respectively while powdered cow dung enhanced the most PAH reduction (252.0 mg kg^{-1}) compared with other organic amendments on 15% SEO contamination level (Table 6).

Table 5. Effect of organic amendments on bacterial polycyclic aromatic hydrocarbons degradation (mg kg^{-1}) at the fifth week.

Organic amendments	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	749.8 ^e	65.9 ^l	585.9 ^h
CPH	499.9 ^j	574.0 ⁱ	806.6 ^d
Cow dung	735.4 ^g	372.8 ^k	749.2 ^f
Control	1066.0 ^c	1726.6 ^b	1865.6 ^a

Note: Means with the same letters in each column are not significantly different using Duncan's multiple range test (DMRT) ($p > 0.05$).

Table 6. Effect of organic amendments on bacterial polycyclic aromatic hydrocarbons degradation (mg kg^{-1}) at the tenth week.

Organic amendments	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	111.6 ^k	55.8 ^l	485.0 ^f
CPH	435.3 ^g	485.9 ^e	520.0 ^d
Cow dung	326.1 ^h	310.5 ⁱ	252.0 ^j
Control	1066.0 ^c	1726.6 ^b	1865.6 ^a

Note: Means with the same letters in each column are not significantly different using Duncan's multiple range test (DMRT) ($p > 0.05$).

Addition of organic amendments to attain significant reduction in the PAH contents of petroleum hydrocarbon related pollution has proved to be successful in this study. The results obtained in this study are in concord with the reports of United State Environmental Protection Agency [USEPA] (1994); Van Gestel, Mergaert, Swings, Coosemans, and Ryckeboer (2003); Ling and Isa (2006); Yerima et al. (2013) on the significance of biostimulating hydrocarbonoclastic bacteria through the adoption of organic amendments with a view to attaining desirable PAH degradation. Since *S. pyogenes* and *E. faecalis* adopted for PAH degradation were isolated from a typical SEO polluted soil, the results obtained in this study are in consonance with the findings of Pothuluri and Cerniglia (1994) on the capability of indigenous microorganisms to utilize PAH contents of petroleum hydrocarbons. The reduction of PAH in terms of its concentrations in the SEO contamination levels using different organic amendments achieved in this study is of significant prominence due to many toxicity and health problems they are associated with.

From the results obtained in this study, a key observation can be seen from the significant reductions of PAH fractions that have been implicated as exerting serious health hazards on the environment. According to Yerima, Umar, Shinkafi, and Ibrahim (2012), these implicated PAH components include Benzo (a) anthracene, Dibenz (a,h) anthracene, Chrysene, Benzo (b) fluoranthene, Indeno (1,2,3 -d) pyrene and Benzo (g,h,i) perylene, Benzo (a) pyrene. It can be seen that the bacterial co-culture coupled with all the organic amendments employed in this study significantly ($p < 0.05$) enhanced their reductions. The biostimulatory influence of the organic amendments in achieving significant reduction in the concentrations of PAH in the SEO contamination levels studied could be attributed to the availability of nutrients needed for its bacterial metabolism.

Conclusion

It can be concluded that the biostimulation of *S. pyogenes* and *E. faecalis* co-culture with compost recorded the most significant TPH reduction (261 mg kg^{-1}) on 10% SEO contamination level. Again, compost enhanced the most significant PAH reduction (55.8 mg kg^{-1}) on 10% SEO contamination level. However, experimental bags that were adopted as control did not record any reduction in the concentrations of hydrocarbon contents shown on the first day of this study. The significant bioremediation capabilities recorded by the bacterial co-culture bioenhanced with organic amendments in this study has made these bioremediation agents potential candidates in remediating soils impacted with petroleum hydrocarbons.

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