Research Article

Biostimulation of Immobilized Microbes in a Fixed-Bed Bioreactor towards Hydrocarbon-Contaminated Water Remediation Using Compost Tea and Egg Shell Powder

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Abstract: The need to satisfy the ever increasing petroleum demand coupled with the availability of enough crude oil reserves to meet demand for several decades to come makes petroleum contamination a globally important subject. Bioremediation has emerged as an efficient treatment option for the remediation of organic contaminants including petroleum owing to the failure of physico-chemical treatment options to address petroleum contamination. The current study targeted the application of compost tea (CT) and egg shell powder (ESP) solution at various levels as nutrient amendments/bio-stimulants for hydrocarbon-eating microbes attached to support media surfaces in a fixed-bed bioreactor system. CT and ESP were characterized to be rich in essential nutrients including N, P, K, Ca, Mg, Na and C. CT and ESP application at four levels each recorded appreciable boosts in microbial numbers and TPH removal across applied levels. CT application recorded 23.49 ± 2.20 to 29.36 ± 0.79 % boost in TPH removal and 17.03 ± 3.01 to 61.07 ± 2.26 % boost in microbial mass. Boosts in TPH removal assumed the order 3200 ml > 1600 ml > 800 ml > 400 ml. ESP application similarly recorded 27.56 ± 2.62 to 29.57 ± 0.90 % boost in TPH removal and 17.50 ± 1.60 to 24.43 ± 2.05 % boost in microbial mass. Recorded TPH removal boosts assumed the trend 20 g/l >10 g/ l > 2.5 g/l >5 g/l.

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1. Introduction

Known to belong to the family of carcinogens and neurotoxins are many hydrocarbon fractions [1-4]. The presence of hydrocarbon contaminants in any given environment thus invariably poses danger to public health and various life forms [4, 5]. Bioremediation presents a viable and efficient technology for the clean-up of hydrocarbon-contaminated environments [6]. Available physico-chemical remediation options such as incineration, burial in secure landfills, etc. aside being expensive and time consuming have only succeeded in relocating the contamination problem often producing toxic products/intermediates that require further disposal or treatment [7-9]. Preference for bioremediation over physico-chemical remediation has largely been due to its environmentally friendly attributes and ability to ensure complete clean-up/removal of contaminants [3, 7, 8].

Restoration of hydrocarbon contaminated media via bioremediation is achieved through the broad biodegradative capabilities of microbial populations adapted to the presence of the hydrocarbon contaminants. Hydrocarbon bioremediation thus essentially exploits the catalytic/enzymatic attributes of adapted microbial populations to convert hydrocarbon contaminants into non-toxic compounds or compounds of relatively low toxicity [3, 7, 10, 11]. Bioremediation largely depends on the enrichment and sustenance of microbial activities in a given environment. The two main approaches to enriching and sustaining microbial activities in a given environment towards degradation of organic contaminants in both aquatic and soil environments are bioaugmentation and biostimulation [3]. Bioaugmentation is often aimed at boosting the numbers of existing microbial populations to hasten the contaminant degradation process. Microbial populations here are either naturally isolated or genetically engineered [12, 13]. Biostimulation on the other hand is targeted at promoting the growth of indigenous microbial populations via processes such as aeration and nutrient addition, among others [3, 13].

Stimulation of the growth of indigenous adapted microbial populations via nutrient application as cited in several studies is crucial to the success of hydrocarbon contaminant remediation in aquatic environments. In limited amounts, nutrients have been implicated to impede microbial hydrocarbon degradation [3, 14, 15]. For purposes of the present study, compost tea and egg shell powder solution were each applied in various amounts as nutrient amendments/bio-stimulants for stimulation of the growth of hydrocarbon-eating microbes immobilized on bamboo support media towards hydrocarbon contaminated water remediation in a locally constructed fixed-bed bioreactor.

2. Materials and methods

2.1 Construction and Operation of Bioremediation Setup

Applied bioremediation setup comprised two sets of reactors in parallel alignment. Each set comprised four reactors connected in series. Incorporated in each vessel was a column for confining applied support media. The approximate volume of each reactor and column was 0.0091 and 0.0024 m^3 respectively. The setup comprised 6 aerobic and 2 anaerobic compartments. The setup operated on two 0.5 hp water pumps each coupled with an electronic timing device. Six cycling regimes were applied per day per experiment with each cycling regime lasting 10 minutes. Flow of sample concentrate from storage tank 1 through reactors into storage tank 2 was achieved at a flow rate of 0.5 L/minute. Sample flow from storage tank 2 into storage tank 1 was at a flow rate of 2 L/minute after every 24 hours. Reactors and columns prior to their application were pre-treated with 10 % NaOCl solution [16, 17] followed by 70 % ethanol solution and finally rinsed with sterile distilled water [16].

2.1.1 Applied Biofilm Support Media

Packed inside each reactor column were bamboo chips (BC) as support media for microbial attachment. Bamboo canes were obtained and chopped into pieces about 2-4 cm in size and washed thoroughly [15]. Bamboo chips were heat sterilized in hot air oven at 180 ^{o}C for 60 minutes and allowed to cool prior to their application [16, 18]. 0.71 kg of bamboo chips was applied per reactor.
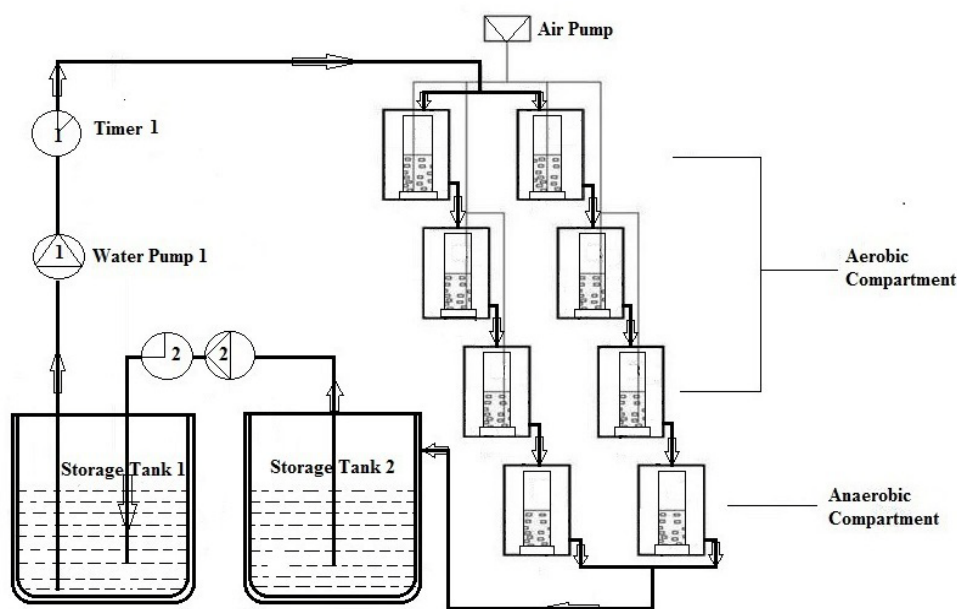

Figure 1 Design of Remediation Setup

2.2 Isolation and Liquid Culturing of Hydrocarbon-Consuming Microbes

To 50 ml of distilled water was added 10 g of homogenized hydrocarbon contaminated soil in a 200 ml beaker. Mixture was stirred for 3 minutes using a magnetic stirrer. 1 ml of the supernatant was pour plated on a mineral salt-agar medium [15]. The plate was supplemented with 0.25 ml of crude oil on setting and allowed to stand for an hour. Plate was incubated at 37 ^{o}C for 72 hours [19, 20]. Sufficient amount of mineral salt solution was used to wash off growths appearing after the 72 hour incubation period into a 1000 ml Erlenmeyer flask. More mineral salt solution was added to make the mark followed by the addition of two drops of crude oil. The mixture was further incubated at 37 ^{o}C for 48 hours [15]. Hydrocarbon contaminated soils were obtained from mechanic workshops located within 'Suame Magazine', a suburb of Kumasi.

2.2.1 Acclimatization of Hydrocarbon-Eating Microbes

Prior to each experimental run, 125 ml of liquid culture of hydrocarbon-eating microbes was poured onto the support media packed inside each reactor column and left to stand for 24 hours with gentle manual agitations at defined intervals. Liquid culture was poured off after the observed 24 hour acclimatization period [15].

2.2.2 Preparation of Mineral Salt Solution and Mineral Salt-Agar Medium

Mineral salt-agar medium was prepared by dissolving 8.0 g of NaCl, 2.5 g of $Na_2HPO_4.2H_2O$, 1.5 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.5 g of $MgSO_4.7H_2O$ and 0.05 g of $FeSO_4.7H_2O$ in a liter of sterile distilled

water [21-23]. To the prepared mineral salt solution was added 20 g of purified agar. The mixture was subjected to heating on a hot plate to aid agar dissolution. The mineral salt-agar medium was autoclaved for 15 minutes at 121 ^{o}C and allowed to cool appreciably before use.

2.3 Hydrocarbon Source

Light sweet crude oil was applied as hydrocarbon source in the present study. Crude oil was obtained from Ghana's Jubilee Oil Field in the Western Region. 1000 mg of crude oil was applied per liter of distilled water.

2.4 Applied Nutrient Amendments

Compost tea (CT) and egg shell powder (ESP) solution were prepared and applied as nutrients/bio-stimulants for hydrocarbon-eating microbes attached to support media surfaces. Compost used in preparing compost tea was obtained from the Accra Compost and Recycling Plant (ACARP). Egg shell powder solution was prepared using fresh hen egg shells obtained from identified vendors within the Ayeduase community near Kwame Nkrumah University of Science and Technology.

2.4.1 Compost Tea (CT) Preparation and Tested Levels

Compost tea was prepared in a 15 L plastic container by adding 500 g of ACARP organic compost to 10 L of distilled water. The mixture was stirred continuously for a couple of minutes. An aquarium air pump was connected to the setup for oxygen supply. The mixture was brewed for 48 hours with intermittent mixing. Brewing was followed by decantation and filtration to obtain compost tea (the water-based fraction) [24]. CT application was at four levels- 400 ml, 800 ml, 1600 ml and 3200 ml. Each level was administered for a week.

2.4.2 Egg Shell Powder (ESP) Solution Preparation and Tested Levels

Egg shells were thoroughly washed with soap and water and air dried. Egg shells were crushed into pieces initially and grinded to fine powder using a mortar and pestle with the aid of liquid nitrogen [25]. ESP levels of 2.5 g/l, 5 g/l, 10 g/l and 20 g/l were applied. Each tested level was brewed for 48 hours prior to application. Each level was administered for a week.

2.4.3 Compost and Egg Shell Powder Characterization

Compost tea and egg shell powder solution were analyzed for their nutritional attributes. The levels of the elements- Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and carbon (C) were estimated. The elements- K, Na, Ca and Mg were determined using the Atomic Absorption Spectrophotometer (AAS). AAS analysis was preceded by elemental extraction/sample digestion. The elements- K, Na, Ca and Mg were extracted from a 25 ml compost tea sample employing the ammonium acetate extraction technique [26, 27]. Wet digestion with nitric acid (HNO_3) and 70 % $HClO_4$ was applied in extracting same elements from a 25 ml egg shell powder sample solution [28]. Available N was determined in a 25 ml sample by the Kjeldahl method. Extractable P was determined in a 25 ml sample using the sodium bicarbonate method as described by [29] and revised by [30]. Organic carbon was determined using Walkley and Black's (1934) wet oxidation method as revised by [31] in a 25 ml sample.

2.5 Enumeration of Hydrocarbon-Eating and Total Heterotrophic Microbes

Hydrocarbon-eating microbes were enumerated via serial dilution and pour plating on a mineral salt agar medium [32, 33]. Plating dishes were heat sterilized in hot air oven for 120 minutes at 180 ^{o}C and allowed to cool appreciably before use. Hydrocarbon-eating microbes attached to support media surfaces were captured in 50 ml distilled water of which 1 ml was serially diluted through to 10^{-19}. 1 ml each of the dilutions 10^{16}-10^{-19} was pour plated in duplicate on mineral salt-agar medium. Plates were supplemented with 0.25 ml crude oil, allowed to stand for an hour and incubated at 37 ^{o}C. Counting of hydrocarbon-eating microbial colonies was performed after 72 hours of incubation.

Total heterotrophic microbial enumeration was by serial dilution and pour plating on nutrient agar medium. Serial dilution followed same procedure as described under hydrocarbon-eating microbial enumeration. Microbes were counted after 48 hours of incubation at 37 ^{o}C [15]. Heterotrophic microbial enumeration was performed for applied compost tea. The average microbial colonies per ml of plated dilutions were estimated using the relation below:

Colony Forming Unit (CFU/ml) = (Number of colonies x Dilution factor)/Volume plated [34, 35].

2.6 Measurement of Total Petroleum Hydrocarbons (TPH)

C_9-C_{36} range of hydrocarbons was measured using a gas chromatograph (GC) coupled with a flame ionization detector (FID) under the following conditions: carrier gas flow rate- 5 ml/minutes, initial temperature- 40 ^{o}C held for 0.5 minutes, program- 40 ^{o}C to 290 ^{o}C at 15 ^{o}C/minute, final temperature- 290 ^{o}C held for 10 minutes, injector temperature- 290 ^{o}C, detector temperature- 300 ^{o}C, make-up gas- 25 ml/minutes. TPH extraction preceded GC-FID analysis. TPH was extracted from a sample volume of 500 ml using 50 ml of methylene chloride. Methylene chloride and water traces were removed from the organic extract using the Soxhlet extractor. Final TPH extract was concentrated in 5ml of methylene chloride and a 2 ml aliquot transferred into a 2 ml vial for GC-FID analysis [15, 36].

3. Results

3.1 Compost Tea (CT) Impact Assessment

The blank study recorded 31.57±2.23 % increase in microbial mass and 70.17±0.94 % TPH removal. Respectively, at applied CT levels of 400, 800, 1600 and 3200 ml, 48.60±0.78, 71.23±1.15, 80.27±1.17 and 92.63±1.50 % increments in microbial mass were recorded. These increments respectively reflected a 17.03±3.01 to 61.07±2.26 % boost in biofilm/microbial mass in relation to the blank. Similarly, TPH removal rates of 93.66±1.95, 96.35±2.09, 97.07±2.35 and 99.53±0.15 % were realized respectively at same applied CT levels, reflecting a 23.49±2.20 to 29.36±0.79 % boost in TPH removal in comparison to the blank [Table 1]. CT performance in terms of TPH removal was of the order 3200 ml > 1600 ml > 800 ml > 400 ml.

One way MANOVA revealed a significant multivariate main effect for CT, Wilks' λ = 0.000, $F_{(8, 16)}$ = 183.499, p = 0.000, partial eta squared = 0.989. Power to detect the effect was 1.000 [Table 2]. Univariate main effect of CT was significant for TPH, $F_{(4, 9)}$ =156.169, p = 0.000, partial eta square = 0.986, power = 1.000 and also significant for MI, $F_{(4, 9)}$ = 827.816, p = 0.000, partial eta square = 0.997, power = 1.000 [Table 3]. Significant pairwise differences were evident for all TPH marginal mean pairs except 400|800, 800|1600, 800|3200 and 1600|3200. Pairwise comparisons for MI means differed significantly (p<0.05) for all pairs. Figures 2a and 2b are respectively bar charts for CT impact

on biofilm/microbial growth and TPH removal. Figure 2c shows the profile plots for TPH and MI marginal means across applied CT levels.

Table 1: Results for Compost Tea (CT) Impact

| CT (ml) | TPH Removal | | Microbial Load Increase | | Performance | |
	ppm	%	CFU/ml	%	TPH (%)	Microbial Increase (MI) (%)
0	6.233±0.083	70.17±0.94	3.37±0.25 E+20	31.57±2.23	0.00±0.00	0.00±0.00
400	8.319±0.173	93.66±1.95	5.20±0.10 E+20	48.60±0.78	23.49±2.20	17.03±3.01
800	8.558±0.185	96.35±2.09	7.50±0.10 E+20	71.23±1.15	26.18±1.88	39.67±2.55
1600	8.622±0.209	97.07±2.35	8.53±0.15 E+20	80.27±1.17	26.90±2.90	48.70±3.40
3200	8.841±0.013	99.53±0.15	9.67±0.21 E+20	92.63±1.50	29.36±0.79	61.07±2.26

Table 2: Multivariate Tests for CT Impact

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[c]
Pillai's trace	1.91	47.751	8	18	0.000	0.955	382.006	1.000
Wilks' lambda	0.000	183.499[a]	8	16	0.000	0.989	1467.996	1.000
Hotelling's trace	772.214	675.687	8	14	0.000	0.997	5405.5	1.000
Roy's largest root	761.939	1714.362[b]	4	9	0.000	0.999	6857.449	1.000

Table 3: Univariate Tests for CT Impact

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[c]
Corrected Model	TPH	1741.167[a]	5	348.233	131.482	0.000	0.986	657.409	1.000
	MI	7267.803[b]	5	1453.561	662.281	0.000	0.997	3311.404	1.000
Intercept	TPH	67.274	1	67.274	25.4	0.001	0.738	25.4	0.994
	MI	32.599	1	32.599	14.853	0.004	0.623	14.853	0.928
AM	TPH	4.528	1	4.528	1.71	0.223	0.160	1.71	0.216
	MI	0.003	1	0.003	0.002	0.970	0.000	0.002	0.050
CT*AM	TPH	1654.477	4	413.619	156.169	0.000	0.986	624.678	1.000
	MI	7267.497	4	1816.874	827.816	0.000	0.997	3311.265	1.000
Error	TPH	23.837	9	2.649					
	MI	19.753	9	2.195					
Total	TPH	126957.4	15						
	MI	70389.85	15						

Corrected Total	TPH	1765.004	14
	MI	7287.556	14

Table 4: Results for Egg Shell Powder (ESP) Impact

ESP (g/l)	TPH Removal		Microbial Load Increase		Performance	
	ppm	%	CFU/ml	%	TPH (%)	Microbial Increase (MI) (%)
0	6.233±0.083	70.17±0.94	3.37±0.25 E+20	31.57±2.23	0.00±0.00	0.00±0.00
2.5	8.720±0.038	98.18±0.43	5.13±0.06 E+20	49.07±1.27	28.01±1.24	17.50±1.60
5	8.680±0.218	97.73±2.46	5.67±0.06 E+20	55.53±1.50	27.56±2.62	23.97±3.31
10	8.761±0.122	98.63±1.37	5.53±0.06 E+20	53.20±0.27	28.46±0.96	21.63±1.97
20	8.859±0.010	99.74±0.11	5.77±0.06 E+20	56.00±0.27	29.57±0.90	24.43±2.05

Table 5: Multivariate Tests for ESP Impact

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[c]
Pillai's trace	1.498	6.708	8	18	0.000	0.749	53.667	0.997
Wilks' lambda	0.002	45.138[a]	8	16	0.000	0.958	361.101	1.000
Hotelling's trace	277.034	242.405	8	14	0.000	0.993	1939.239	1.000
Roy's largest root	276.029	621.065[b]	4	9	0.000	0.996	2484.26	1.000

Similarly, ESP at applied levels of 2.5 g/l, 5 g/l, 10 g/l and 20 g/l, respectively recorded 49.07±1.27, 55.53±1.50, 53.20±0.27 and 56.00±0.27 % increments in microbial mass reflecting 17.50±1.60 to 24.43±2.05 % boost in microbial mass over the blank. TPH removal rates at the same applied levels were respectively 98.18±0.43, 97.73±2.46, 98.63±1.37 and 99.74±0.11 %, reflecting 27.56±2.62 to 29.57±0.90 % boost in TPH removal over the blank [Table 4]. Significant increases in microbial mass and TPH removal rates in relation to the blank were thus evident across applied ESP levels.

A significant multivariate main effect for ESP was observed from one way MANOVA analysis, -Wilks' $\lambda = 0.002$, $F_{(8, 16)} = 45.138$, $p = 0.000$, partial eta squared = 0.958, power = 1.000 [Table 5]. Univariate main effects of ESP were significant for TPH, $F_{(4, 9)} = 294.529$, $p = 0.000$, partial eta square = 0.992, power = 1.000 and MI, $F_{(4, 9)} = 190.402$, $p = 0.000$, partial eta square = 0.988, power = 1.000 [Table 6]. Pair-wise comparisons among TPH marginal means differed significantly ($p<0.05$) for all pairs except- 2.5|5, 2.5|10, 2.5|20, 5|10, 5|20 and 10|20. Similarly, pair-wise comparisons among MI marginal means for all pairs were significantly different ($p<0.05$) except for the pairs 5|10, 5|20 and 10|20. Figures 3a and 3b are respectively bar charts for ESP impact on biofilm/microbial growth and TPH removal. Figure 3c shows the profile plots for TPH and MI marginal means across applied ESP levels. Tables 7 and 8 respectively represent results for monitored parameters for CT and ESP studies. Table 9 shows results for nutritional and microbial load of CT and ESP.
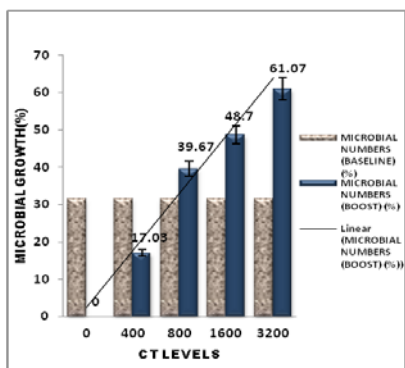
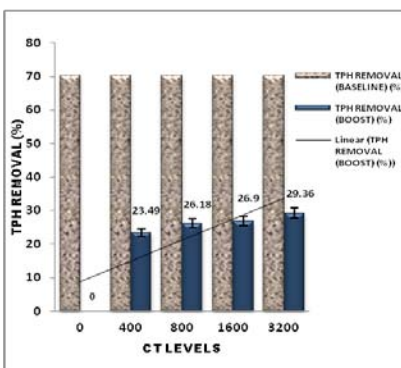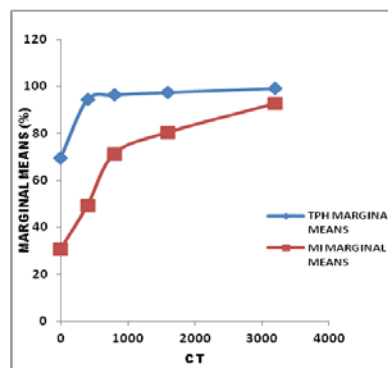**Figure 2a:** CT Impact (Microbial Growth)   **Figure 2b:** CT Impact (TPH Removal)   **Figure 2c:** Profile plots for TPH and MI Marginal means
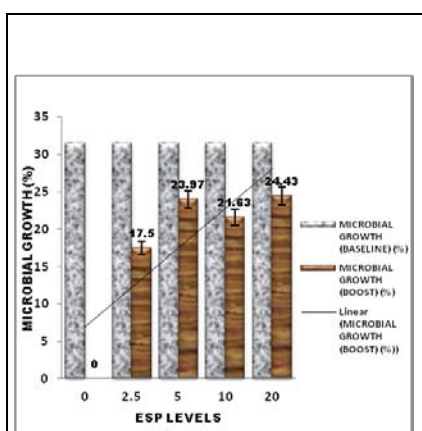

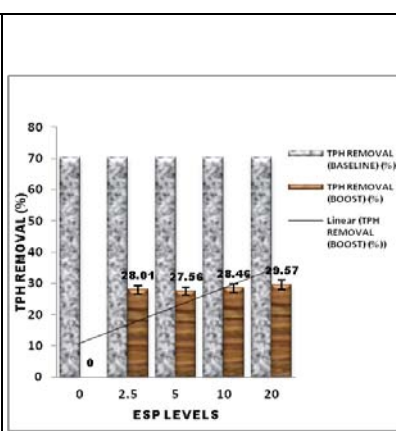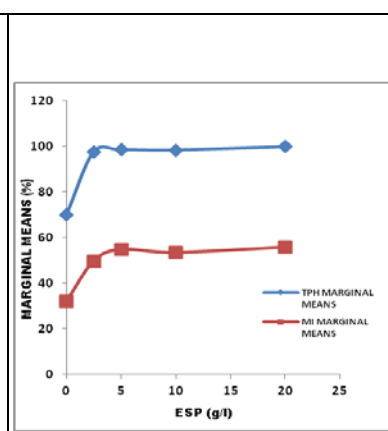**Figure 3a:** ESP Impact (Microbial Growth)   **Figure 3b: ESP Impact (TPH Removal)**   **Figure 3c:** Profile plots for TPH and MI Marginal means

Table 6: Univariate Tests for ESP Impact

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[c]
Corrected Model	TPH	1945.615[a]	5	389.123	239.706	0.000	0.993	1198.531	1.000
	MI	1244.777[b]	5	248.955	174.873	0.000	0.990	874.367	1.000
Intercept	TPH	3.514	1	3.514	2.165	0.175	0.194	2.165	0.261
	MI	18.856	1	18.856	13.245	0.005	0.595	13.245	0.898
AM	TPH	4.265	1	4.265	2.628	0.139	0.226	2.628	0.305
	MI	4.534	1	4.534	3.185	0.108	0.261	3.185	0.358
ESP*AM	TPH	1912.477	4	478.119	294.529	0.000	0.992	1178.118	1.000
	MI	1084.251	4	271.063	190.402	0.000	0.988	761.609	1.000
Error	TPH	14.61	9	1.623					

	MI	12.813	9	1.424
Total	TPH	131384.8	15	
	MI	37380.47	15	

Table 7: Results for Monitored Parameters- CT Study

CT (ml)	PARAMETER	DAY 1	DAY 3	DAY 5	DAY 7
0	**pH**	7.06±0.05	7.32±0.03	7.33±0.08	7.30±0.02
	D.O. (mg/l)	0.79±0.02	1.36±0.04	1.80±0.09	2.19±0.16
	Temperature (^{o}C)	26.17±0.55	27.00±0.36	27.00±0.76	27.57±0.40
	Microbial Count (CFU/ml)	1.07±0.04E+21			1.40±0.05E+21
400	**pH**	7.02±0.02	7.10±0.05	7.18±0.07	7.17±0.11
	D.O. (mg/l)	0.81±0.03	1.37±0.05	1.80±0.06	2.34±0.10
	Temperature (^{o}C)	25.43±0.47	25.27±0.50	25.40±0.53	26.33±0.12
	Microbial Count (CFU/ml)	1.07±0.03E+21			1.59±0.04E+21
800	**pH**	7.07±0.03	7.19±0.12	7.29±0.03	7.30±0.06
	D.O. (mg/l)	0.85±0.02	1.37±0.03	1.75±0.04	2.33±0.10
	Temperature (^{o}C)	26.57±1.01	26.93±0.32	26.83±0.65	26.93±0.15
	Microbial Count (CFU/ml)	1.05±0.02E+21			1.80±0.03E+21
1600	**pH**	7.02±0.04	7.04±0.06	7.23±0.12	7.27±0.07
	D.O. (mg/l)	0.84±0.03	1.40±0.03	1.79±0.04	2.35±0.06
	Temperature (^{o}C)	27.43±0.50	28.33±0.51	28.37±1.07	28.80±0.78
	Microbial Count (CFU/ml)	1.06±0.04E+21			1.92±0.05E+21
3200	**pH**	7.03±0.05	7.14±0.14	7.15±0.15	7.24±0.13
	D.O. (mg/l)	0.86±0.02	1.39±0.02	1.79±0.02	2.36±0.05
	Temperature (^{o}C)	27.70±0.46	28.13±0.47	28.53±0.55	28.50±0.20
	Microbial Count (CFU/ml)	1.04±0.02E+21			2.01±0.03E+21

Table 8: Results for Monitored Parameters- ESP Study

ESP (g/l)	PARAMETER	DAY 1	DAY 3	DAY 5	DAY 7
0	**pH**	7.06±0.05	7.32±0.03	7.33±0.08	7.30±0.02
	D.O. (mg/l)	0.79±0.02	1.36±0.04	1.80±0.09	2.19±0.16
	Temperature (^{o}C)	26.17±0.55	27.00±0.36	27.00±0.76	27.57±0.40
	Microbial Count (CFU/ml)	1.07±0.04E+21			1.40±0.05E+21
2.5	**pH**	7.04±0.07	7.15±0.05	7.24±0.12	7.28±0.05
	D.O. (mg/l)	0.87±0.02	1.35±0.04	1.82±0.03	2.37±0.10

	Temperature (ºC)	25.27±0.47	25.73±1.19	26.00±0.36	26.60±0.66
	Microbial Count (CFU/ml)	1.05±0.02E+21			1.56±0.01E+21
5	pH	7.08±0.05	7.23±0.08	7.27±0.05	7.35±0.05
	D.O. (mg/l)	0.88±0.02	1.32±0.04	1.83±0.05	2.27±0.09
	Temperature (ºC)	25.83±0.68	26.03±0.90	26.50±0.40	26.23 ±0.51
	Microbial Count (CFU/ml)	1.02±0.02E+21			1.59±0.01E+21
10	pH	7.03±0.04	7.13±0.13	7.30±0.03	7.26±0.08
	D.O. (mg/l)	0.87±0.01	1.34±0.03	1.80±0.03	2.36±0.07
	Temperature (ºC)	27.83±0.61	28.17±0.61	28.00±1.51	28.23±0.59
	Microbial Count (CFU/ml)	1.04±0.01E+21			1.59±0.02E+21
20	pH	7.07±0.04	7.16±0.07	7.20±0.08	7.26±0.06
	D.O. (mg/l)	0.80±0.03	1.29±0.02	1.61±1.68	2.27±0.07
	Temperature (ºC)	27.63±1.50	28.60±1.57	28.40±0.70	28.67±1.36
	Microbial Count (CFU/ml)	1.03±0.01E+21			1.61±0.01E+21

Table 9: Results for Nutritional and Microbial Load of CT and ESP

PARAMETER	APPLIED AMENDMENT	
	CT	ESP
Na (mg/l)	8.77±0.06	8.14±0.10
Mg (mg/l)	5.40±0.03	4.37±0.03
Ca (mg/l)	15.40±0.10	39.56±1.24
K (mg/l)	10.25±0.01	5.14±0.05
P (mg/l)	26.74±0.32	9.10±0.03
C (%)	5.94±0.05	2.21±0.12
N (%)	0.86±0.03	1.01±0.13
Microbial Count (CFU/ml)	1.24±0.03E+20	-

4. Discussion

Compost tea application in general was characterised by increases in microbial growth and TPH removal as CT levels increased or were doubled. The correspondence between increases in microbial numbers and TPH removal was to be expected. The correspondence typically suggests hydrocarbon-eating microbes were actively making use of CT as a nutrient source and applied oil as the only carbon source [15]. The order of CT performance was 3200 ml > 1600 ml > 800 ml > 400 ml. The sharp increases in microbial mass as CT volumes doubled (Figure 2a) in comparison to the blank, could

as well be attributed to the possible introduction of significant numbers of hydrocarbon-eating microbes proportional to the applied volumes to augment the numbers originally introduced [14, 37].

Compost tea was rich in essential nutrients including N, P, K, Ca, Mg, Na and C [Table 9, 38, 39] which have been established to be crucial to the survival of hydrocarbon-eating microbes in several studies [14, 40, 41, 42]. Increments in microbial counts suggest the availability and effective use of these essential elements in proportions favourable to the growth of hydrocarbon-eating microbes. High proportions of these nutrients would have been detrimental to hydrocarbon-eating microbes via toxic effects [14, 40, 41]. TPH removal rates are typically indicative of the adaptive response of hydrocarbon-eating microbes to the presence of the applied oil. TPH removal rates recorded were essentially a function of the significant interaction effect between applied CT levels and immobilized hydrocarbon-eating microbes as realized statistically ($p<0.05$) [Table 3].

ESP similarly at all levels of application triggered appreciable boosts in biofilm/microbial mass and TPH removal rates. However, increments in microbial mass and TPH removal assumed different patterns across applied levels. The respective trends were 20 g/l >5 g/l > 10 g/l >2.5 g/l and 20 g/l > 10 g/l > 2.5 g/l >5 g/l. Trends typically did not give a true reflection of the actual numbers of hydrocarbon-eating microbes involved in the hydrocarbon degradation process, an observation similar to that made by [15]. It is also possible that there was a time lapse in microbial acclimatization to reactor conditions and the presence of the hydrocarbon contaminant.

Hen egg shells according to Siulapwa et al. (2014) and Yasothai and Kavithaa, (2014) are rich sources of essential nutrients. Characterization of powdered hen egg shells confirmed the presence of appreciable levels of essential nutrients- N, P, K, Ca, Mg, Na and C [Table 9]. Boosts in microbial counts and TPH removal rates are suggestive of the availability and utilization of the abovementioned essential elements in amounts favourable to the survival of hydrocarbon-eating microbes and also indicative of significant interaction effect between applied ESP levels and hydrocarbon-eating microbes. Thus a significant interaction effect between applied ESP levels and immobilized hydrocarbon-eating microbes cannot be ruled out ($p<0.05$) to have immensely contributed to the recorded boosts in microbial counts and the corresponding TPH removal rates [Table 6]. High nutrient levels as explained by [14], [40], [41] and [42] and others would have impeded hydrocarbon degradation via toxicity effects on hydrocarbon-eating microbes.

At higher CT and ESP levels, the rate of uptake and mineralization of applied oil by hydrocarbon-eating microbes was expected to be hampered by virtue of the presence of C in both CT and ESP. This was thus anticipated to present microbes with a dual carbon source for utilization. Nonetheless, relatively high TPH removal rates across all levels of CT and ESP application were recorded, suggestive of the preference of hydrocarbon-eating microbes for the applied oil as main carbon source. Thus the C in the applied amendments possibly only had minimal/negligible impact on the hydrocarbon degradation process an observation similar to that of [45] who found the impact of supplementary C to be minimal on the degradation of PAH's.

The performances observed for the applied amendments in the current study (> 93 %) are quite consistent with the performance of conventional mineral salt medium applied in a similar study by [15] in which they recorded 36.83 to 93.86 % TPH removal.

Biofilm support media typically offered surfaces for attachment of microbial cells via adsorption. Microbes have the natural tendency to adhere to surfaces of support media by adsorption via van der Waal forces, ionic and hydrophobic interactions as well as hydrogen bonding to form biofilms [46-48]. Adsorption typically facilitated direct contact between hydrocarbon-eating microbes and hydrocarbons

as well as available nutrients within the medium [47, 49] resulting in appreciable TPH degradation rates as recorded.

Hydrocarbon contaminant bioremediation in a given environment as evident from several studies is largely dependent on the establishment of conditions supportive of the growth and survival of hydrocarbon-eating microbial populations at play in the remediation process [3, 14]. Aside the need for adequate nutrients by hydrocarbon-eating microbes, adequate oxygen levels (in the case of aerobic biodegradation) as well as suitable pH and temperature conditions have equally been implicated to be crucial to hydrocarbon degradation [3, 14, 50]. As evident from the present study, CT impact manifested over a pH range of 7.02±0.02 to 7.33±0.08, D.O range of 0.79±0.02 to 2.36±0.05 mg/l and temperature range of 25.27±0.47 to 28.80±0.78 ^{o}C. ESP impact similarly manifested over respective optimum pH, D.O and temperature ranges of 7.03±0.04 to 7.35±0.05, 0.79±0.02 to 2.37±0.10 mg/l and 25.27±0.47 to 28.67±01.36 ^{o}C.

The recorded pH ranges are suggestive of hydrocarbon-eating bacteria as the principal hydrocarbon degraders in the present study. Near neutral pH conditions as cited by [14] are more favourable to hydrocarbon-eating bacteria with acidic conditions favouring hydrocarbon-eating fungi. Relatively higher hydrocarbon removal rates have been reported at near neutral pH conditions [40, 51 52] predominantly at slightly alkaline pH conditions [13, 53, 54, 55]. The relatively high TPH removal rates achieved in the present study were thus to be expected at the recorded near neutral pH ranges. The current study is consistent with the generally accepted view that hydrocarbon-eating microbes have a strong inclination towards near neutral pH conditions [14]. The observation by [56] relating slightly acidic conditions to the production of acidic intermediates suggests that the slightly alkaline conditions observed were indicative of the production of alkaline intermediates.

The study recorded high TPH removal rates over high D.O. levels for both CT and ESP applications, an observation consistent with that of [15] who reported of TPH removal rates of 36.83 to 93.86 % over a D.O range of 2.29 to 3.62 mg/l. The correspondence between increasing D.O levels and increasing TPH removal rates is suggestive of the active utilization of the supplied oxygen by aerobic hydrocarbon-eating microbes actively involved in the degradation of hydrocarbons within the aerobic reactors [14, 50].

Optimum hydrocarbon degradation according to [57] typically occurs in the temperature range of 20 to 35 °C. This is consistent with the findings of [42] who also reported of a temperature range of 20 to 30 °C to be favourable to hydrocarbon degradation in freshwater environments. The ambient temperature to which the present study was subjected typically influenced the temperature ranges recorded. Ambient temperature typically may have exerted its influence on the physical nature of the oil contaminant (ensuring the applied oil remained in the liquid state) as well as the makeup and metabolism of the hydrocarbon-eating microbial communities at play [13, 14, 40, 50]. The recorded temperature ranges are suggestive of the involvement of psychrotrophic and mesophilic hydrocarbon-eating microbes [50] in the degradation process. With reference to the relatively high TPH removal rates recorded, it can be inferred that the recorded temperature ranges were quite favourable to the metabolic/enzymatic activities of the implicated hydrocarbon-eating microbes. It is also possible that ambient temperatures were high enough to facilitate non-biological losses via volatilization/evaporation [13, 14, 40, 58].

5. Conclusion

The application of compost tea and egg shell powder as nutrient amendments for hydrocarbon-eating microbes towards hydrocarbon degradation in a water medium was successful. Compost tea and egg shell powder stimulated appreciable increases in microbial numbers which translated into appreciable TPH removal rates. Thus significant interaction effect existed between hydrocarbon-eating microbes (attached to support substrate surfaces) and the applied nutrient amendments to ensure the survival of hydrocarbon-eating microbes towards hydrocarbon degradation. The technique proved efficient in treating hydrocarbon contaminated water considerably.

Acknowledgments

Author Contributions

Samuel Antwi-Akomeah was the principal investigator, conceived and modelled the applied remediation setup, designed and performed experiments and wrote the paper; **Dr. Bernard Fei-Baffoe** conceived the design of the remediation setup, supervised the conduct of experiments and assisted in the review of manuscript/paper; **Dr. Ebenezer J.D. Belford** assisted in the supervision and conduct of experiments and review of manuscript/paper; **Dr. Paul Osei-Fosu** assisted in TPH analysis and interpretation of results.

Conflicts of Interest

The authors declare no conflict of interest.

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