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Impact Analysis of Cow and Goat Biles as Amendments in the Remediation of Petroleum Contaminated Water Using a Fixed-Bed Bioreactor Setup

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Abstract:

Petroleum remains a major source of energy for industry and daily life. There is enough oil reserve to sustain the trend for decades to come. The quest to meet the increasing global demand for petroleum has been associated with proportional rise in hydrocarbon contamination. Bioremediation has received favourable consideration as an efficient means to dealing with the problem of petroleum contamination. The performance of cow bile (CB), goat bile (GB) and a 1:1 mix of both biles at various levels as bio-stimulants towards the remediation of hydrocarbon contaminated water were tested in the current study. Applied biles stimulated appreciable boosts in microbial mass and TPH removal. CB+GB mix emerged the best performing bile at all levels in terms of its impact on TPH removal and microbial mass. CB, GB and CB+GB application respectively registered 10.56±1.63 to 16.47±0.21 %, 21.08±3.01 to 27.39±0.49 % and 24.56±0.49 to 29.19±1.06 % boosts in TPH removal and 5.05±1.63 to 10.25±1.91 %, 3.80±1.27 to 9.10±2.12 % and 6.55±1.77 to 18.30±3.11 % boosts in microbial mass. TPH removal and microbial mass boosts across applied levels assumed the trend 40>20>10 for all biles. A significant interaction effect existed between applied bile levels and immobilized hydrocarbon-eating microbes statistically (p>0.05).

Keywords
Biostimulation; Bioremediation; Fixed-bed bioreactor; Hydrocarbon-eating microbes; Petroleum contamination; Total Petroleum Hydrocarbon; Support media; Cow bile; Goat bile.

1. Introduction

Petroleum remains the most consumed of all the primary energy sources and accounts for over 36 % of hydrocarbon fuels [1]. The quest to satisfy the surging demand worldwide makes petroleum contamination of aquatic and soil media a globally important subject that deserves attention [2, 3, 4]. Petroleum has often found its way into water and soil media via accidental spills and leakages. Deliberate disposal practices have also been cited as another major route of entry of hydrocarbons chiefly waste motor oil into both aquatic and soil media [5, 6]. Out of the huge quantum entering aquatic and soil media, one gallon of petroleum or its product is enough to render one million gallons of freshwater contaminated and about four acres of fertile land infertile for decades [7]. Hydrocarbon contaminants thus constitute public and socio-economic threats to life forms taking into account the carcinogenic, neurotoxic and mutagenic attributes of several hydrocarbon fractions [4, 8, 9, 10].

Several treatment options exist for the remediation of hydrocarbon contaminated media including physico-chemical and biological treatment options. Physico-chemical remediation options include incineration and burial in secure landfills among others. Aside being expensive and time consuming, physico-chemical treatment techniques have often been associated with the production of intermediates or products of considerable toxicity that may require further treatment or disposal [11-13]. Hydrocarbon contaminated media restoration via bioremediation is a function of the broad exploits the catalytic/enzymatic capabilities of adapted microbial populations to convert hydrocarbon contaminants into non-toxic compounds biodegradative attributes of microbial populations adapted to the presence of the hydrocarbon contaminant. Bioremediation thus essentially

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or relatively less toxic compounds [4, 11, 14]. Its environmentally friendly attribute in addition to being able to render complete breakdown and/or removal of hydrocarbon contaminants makes it a more preferred remediation option over its physico-chemical remediation counterparts [4, 11, 12]. The ability to create and maintain conditions favourable to the metabolic activities of adapted microbial populations in a given environment is crucial to the success of bioremediation. The main approaches to enriching and sustaining microbial activities in a given environment towards hydrocarbon bioremediation are bioaugmentation and biostimulation [4].

Bioavailability of nutrients and surfactant activity are pivotal to the success of hydrocarbon contaminant remediation in aquatic environments. Nutrients, particularly nitrogen and phosphorus have been cited to hamper the microbial degradation of hydrocarbons in limited amounts [15]. Microbially induced surfactants as studies have it, also aid the breakdown and dispersal of petroleum thus enhancing the bioavailability of hydrocarbons for uptake by hydrocarbon-eating microbes [16-18]. The present study in this regard tested the nutritional and surfactant attributes of cow and goat biles and a 1:1 mix of both biles at various levels towards hydrocarbon contaminated water remediation in a locally modelled fixed-bed bioreactor.

Support Material Applied

Bamboo chips were applied as biofilm support material for microbial attachment within the columns. Bamboo chips were about 2-4 cm in size.

2. Methodology

Applied Bioremediation Setup

Bioremediation setup [Figure 1] comprised a total of eight cylindrical reactors sorted into two sets of four reactors each. Reactors in each set were serially connected to one another with the two reactor sets in parallel alignment. Inside each reactor was a column into which was packed biofilm support materials. 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 aerobic reactors were aerated with aquarium pumps. Two 0.5 hp water pumps each coupled with an electronic timing device were applied in operating the setup. Six cycling periods each lasting for 10 minutes were applied per day per experiment. A flow rate of 0.5 L/minute was administered for sample flow from storage tank 1 through reactors into storage tank 2. Sample flow from storage tank 2 into storage tank 1 was achieved at an administered flow rate of 2 L/minute every 24 hours. A sample concentration of 1000 mg/l was administered per experiment. Reactors and columns prior to application were thoroughly rinsed with10 % NaOCl solution [19, 20] followed by 70 % ethanol solution and finally with distilled water [19].

They were thoroughly washed and subjected to dry heat sterilization in hot air oven at 180 ^oC for 60 minutes and allowed to cool prior to their application [19, 20]. 0.71 kg of bamboo chips was applied in each reactor column.

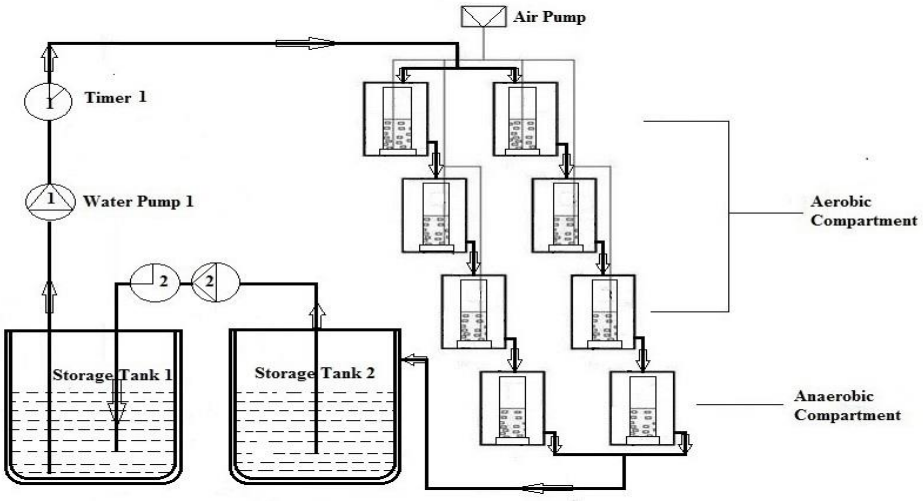

Figure 1: Configuration of Remediation Setup

Mineral Salt Solution and Mineral Salt-Agar Medium Preparation

The recipes of [22-24] were combined to produce a mineral salt solution containing 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 distilled water. The solution was stirred to facilitate dissolution of the salts. Mineral salt-agar medium was prepared by dissolving 20 g of purified agar in a liter of mineral salt solution (prepared as above). The mixture was heated on a hot plate to hasten agar dissolution followed by autoclaving at 121 ^oC for 15 minutes. Mineral salt-agar medium was allowed to cool appreciably prior to use.

Liquid Culturing of Hydrocarbon-Eating Microbes

Hydrocarbon-eating microbes were isolated from hydrocarbon contaminated soils fetched from mechanic workshops within 'Suame Magazine', a suburb of Kumasi. Soils were sampled at three different spots from each of three identified garages into petri dishes and transported to the laboratory where they were homogenized. In a 200 ml beaker was transferred 10 g of homogenized hydrocarbon contaminated soil followed by the addition of 50 ml of distilled water. Using a magnetic stirrer the mixture was stirred for 3 minutes. 1ml of the resulting solution was pour plated on a mineral salt-agar medium [5]. On setting, 0.25 ml of crude oil was spread on the surface of the inoculated mineral salt-agar medium and allowed to stand for about one hour. The plate was incubated for 72 hours at 37 ^oC [25, 26**]**. Sufficient amount of mineral salt solution was used to wash off growths appearing at the end of the incubation period into a 1000 ml Erlenmeyer flask. Additional mineral salt solution was added to make the mark. 2 drops of crude oil was added to the liquid culture and further incubated at 37 ^oC for 48 hours [5].

Acculturation of Hydrocarbon-Eating Microbes to Reactor Conditions

Preceding each experiment, hydrocarbon-eating microbes were exposed to reactor conditions. Unto the biofilm support media packed inside each column was added 125 ml of liquid culture of hydrocarbon-eating microbes. Columns were left to stand for 24 hours with gentle manual agitation at defined intervals. Liquid culture was poured off after the 24 hour period [5].

Applied Hydrocarbon and Tested Concentration

Hydrocarbon source was light sweet crude oil obtained from Ghana's Jubilee Oil Field in the Western Region. Crude oil was autoclaved at 121 ^oC for 15 minutes [19]. 1000 mg of crude oil was applied per liter of distilled water.

Applied Biles and Levels Tested

Cow and goat biles were applied for their nutritional and surfactant impacts on hydrocarbon degradation. Applied biles were obtained from Johnny's Food and Meat Complex (JFAMCO) abattoir in Madina, a suburb of Accra. Biles were tested for their individual impact as well as their combined (1:1) impact on hydrocarbon degradation. Cow and goat biles as well as the 1:1 mix of both biles were applied at three levels each for their impact on hydrocarbon degradation. Tested levels were- 10, 20 and 40 ml.

Characterization of Cow and Goat Biles

Applied biles were characterized for the levels of the elements- Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and carbon (C). Available N was determined in a 25 ml sample volume by the Kjeldahl method [28]. Extractable P was similarly determined in a 25 ml sample using the sodium bicarbonate method as described by [27] and revised by [28]. Organic C was determined by the wet oxidation method [29, 30] in a 25 ml sample. The levels of K, Na, Ca and Mg were estimated via Atomic Absorption Spectrophotometry (AAS). AAS analysis was preceded by sample digestion with 25 ml concentrated nitric acid (HNO_3) and 10 ml 70 % $HClO_4$ in a digestion vessel. The digestate was filtered using Whatman No.41 filter paper into a 100 ml volumetric flask and brought to volume with distilled water [31, 32].

Enumeration of Hydrocarbon-Eating and Total Heterotrophic Microbes

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Enumeration of hydrocarbon-degrading microbes was by serial dilution and pour plating on a prepared mineral salt-agar medium [33, 34]. Petri-dishes prior to use were heat sterilized in hot air oven at 180 ºC for 2 hours. They were allowed to cool appreciably prior to use. Biofilm on support media surfaces were captured in 50 ml distilled water and 1ml of it serially diluted through to 10^{-19}. 1 ml of each of the dilutions 10^{16}-10^{-19} was pour plated in duplicate on a mineral salt-agar medium. Plates were fortified with 0.25 ml crude oil as carbon source and allowed to stand for 60 minutes. Plates were incubated at 37 ºC for 72 hours.

Enumeration of total heterotrophic microbes in applied biles was similarly by serial dilution and pour plating on nutrient agar medium. Serial dilution followed same procedure as described under hydrocarbon-eating microbial enumeration. The plates were incubated for 48 hours at 37 ºC. 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 [35, 36].

Estimation of Total Petroleum Hydrocarbon (TPH)

A gas chromatograph (GC) coupled with a flame ionization detector (FID) was used in measuring TPH levels per the method described by the Environmental Research Institute, University of Connecticut. The following GC conditions were adhered to according to the adopted methodology: carrier gas flow rate- 5ml/minutes, initial temperature- 40 ºC held for 0.5 minutes, program- 40 ºC to 290 ºC at 15 ºC/minute, final temperature- 290 ºC held for 10 minutes, injector temperature- 290 ºC, detector temperature- 300 ºC and make-up gas- 25 ml/minutes. The method applied to C_9-C_{36} range of hydrocarbons. Preceding GC-FID analysis was TPH extraction. TPH was extracted from a sample volume of 500 ml with 50 ml methylene chloride. The extract was subjected to Soxhlet extraction to rid it of methylene chloride solvent and water traces. Final TPH extract was concentrated in 5 ml of methylene chloride and a 2 ml aliquot transferred into a 2 ml vial for GC-FID analysis [5, 37].

3. Results

At applied levels of 10, 20 and 40 ml, relatively higher increases in microbial mass and TPH removal rates were evident. The blank study yielded 31.00±2.83 % increase in microbial mass and 70.19±1.32 % TPH removal. Microbial mass increments in comparison to the blank reflected performance boosts of- 5.05±1.63 to 10.25±1.91 %, 3.80±1.27 to 9.10±2.12 % and 6.55±1.77 to 18.30±3.11 % respectively for CB, GB and CB+GB. In a similar manner, THP removal rates realized with reference to the blank also reflected performance boosts of- 10.56±1.63 to 16.47±0.21 % for CB, 21.08±3.01 to 27.39±0.49 % for GB and 24.56±0.49 to 29.19±1.06 % for CB+GB across applied levels [Table 1].

Table 1: Results for Bile Impact

Bile (ml)	TPH Removal	Microbial Load Increment	Performance	
	%	%	TPH (%)	Microbial Increase (MI) (%)
B@0	70.19±1.32	31.00±2.83	0.00±0.00	0.00±0.00
CB@10	80.75±0.30	36.05±1.20	10.56±1.63	5.05±1.63
CB@20	85.07±2.57	37.00±2.12	14.89±1.25	6.00±0.71
CB@40	86.65±1.53	41.25±0.92	16.47±0.21	10.25±1.91
GB@10	91.27±4.33	34.80±1.56	21.08±3.01	3.80±1.27
GB@20	97.57±0.83	36.15±0.50	27.39±0.49	5.15±2.33
GB@40	97.13±3.34	40.10±0.71	26.94±2.02	9.10±2.12
CB+GB@10	94.74±0.83	37.55±1.06	24.56±0.49	6.55±1.77
CB+GB@20	99.01±0.28	46.35±1.06	28.83±1.04	15.35±1.77
CB+GB@40	99.38±0.26	49.30±0.28	29.19±1.06	18.30±3.11

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Illustrated respectively in figures 2a and 2b below are biofilm/microbial increments and TPH removal rates recorded for the different biles at various levels.

Figures 2c, 2d and 2e respectively represent TPH and MI marginal means profile plots for CB, GB and CB+GB.

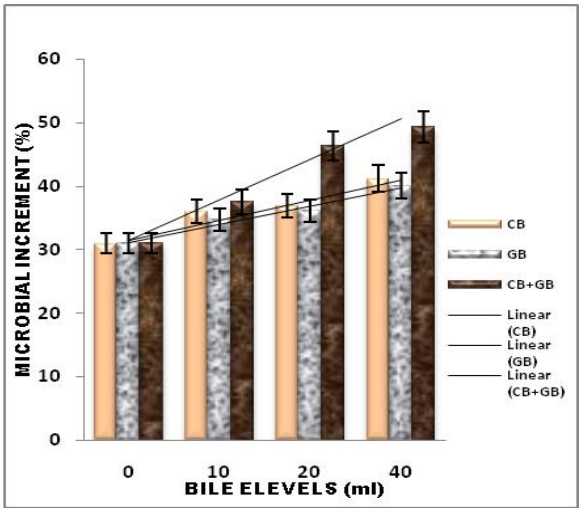

Figure 2a: Bile Impact (Microbial Growth)

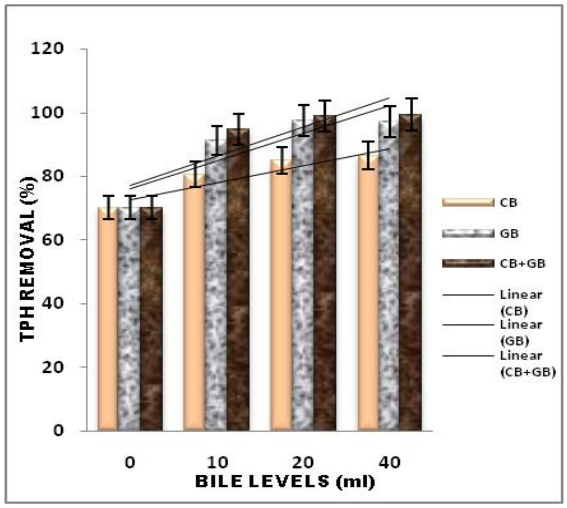

Figure 2b: Bile Impact (TPH Removal)

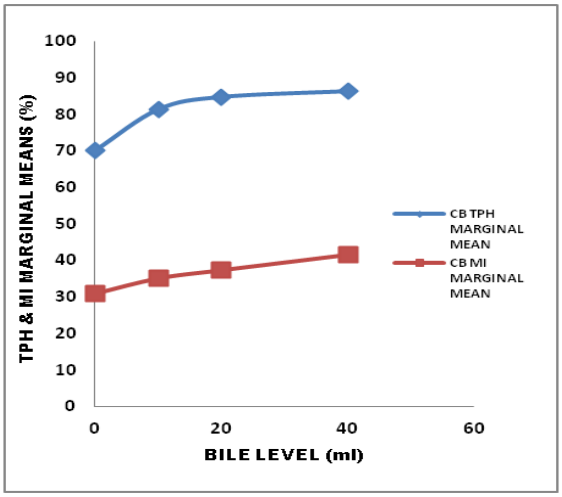

Figure 2c: TPH and MI Profile plot for CB

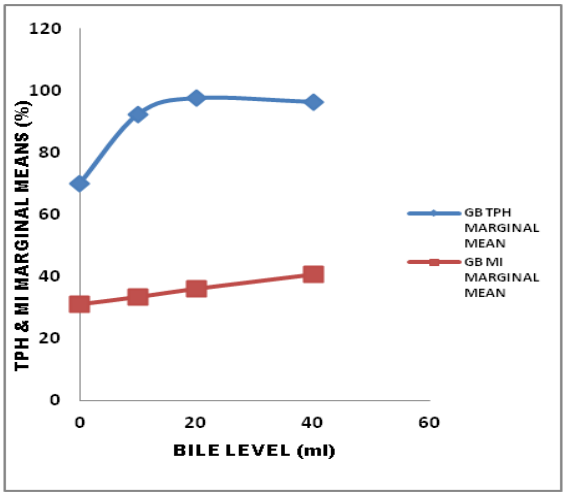

Figure 2d: TPH and MI Profile plot for GB

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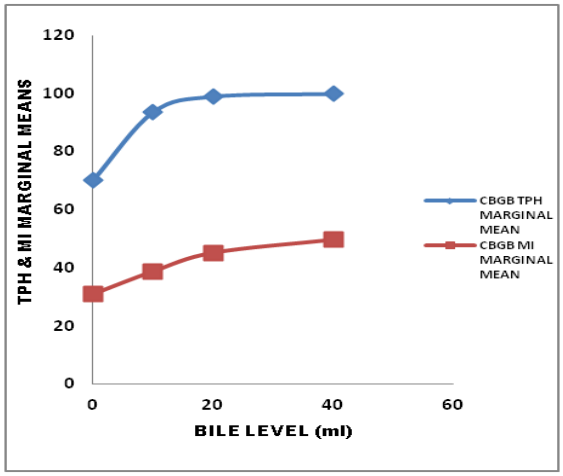

Figure 2e: TPH and MI Profile plot for CB+GB

One way MANOVA analysis conducted revealed significant multivariate main effect for applied biles- Wilks' λ = 0.000, F (22, 20) =73.258, p = 0.000, partial eta squared = 0.988. Effect detection power was 1.000 [Table 2]. Also, univariate main effects of biles were significant for TPH, F (11, 11) = 89.178, p = 0.000, partial eta square = 0.989, power = 1.000 and MI, F (11, 11) = 100.152, p = 0.000, partial eta square = 0.990, power = 1.000 [Table 3]. Pair-wise comparisons among marginal means for TPH removal differed significantly except ($p<0.05$) for the pairs- CB10|CB20, CB20|CB40, GB10|GB40, GB10|CBGB10, GB20|GB40, GB20|CBGB10, GB20|CBGB20, GB20|CBGB40, GB40|CBGB20, GB40|CBGB40 and CBGB20|CBGB40. For MI means, pair-wise comparisons revealed significant differences except for the pairs- CB10|CB20, CB10|GB10, CB10|GB20, CB20|GB20, CB40|GB40, GB10|GB20 and CB20|CBGB10.

Significant interaction effect ($p<0.05$) existed between the applied bile levels and immobilized hydrocarbon-eating microbes (BILE*AM) [Table 3].

Table 2: Multivariate Test for Bile Impact

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	1.922	24.744	22	22	0.000	0.961
Wilks' lambda	0.000	73.258[a]	22	20	0.000	0.988
Hotelling's trace	515.081	210.715	22	18	0.000	0.996
Roy's largest root	502.872	502.872[b]	11	11	0.000	0.998

Table 3: Univariate Test for Bile Impact

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	TPH	2974.010[a]	12	247.834	82.483	0.000	0.989
	MI	781.417[b]	12	65.118	93.359	0.000	0.990
Intercept	TPH	0.634	1	0.634	0.211	0.655	0.019
	MI	51.346	1	51.346	73.614	0.000	0.870
MC	TPH	21.824	1	21.824	7.263	0.021	0.398
	MI	24.035	1	24.035	34.459	0.000	0.758
BILE*AM	TPH	2947.479	11	267.953	89.178	0.000	0.989
	MI	768.412	11	69.856	100.152	0.000	0.990
Error	TPH	33.051	11	3.005			
	MI	7.673	11	0.698			
Total	TPH	184004.2	24				
	MI	34771.99	24				
Corrected Total	TPH	3007.061	23				
	MI	789.09	23				

Table 4: Results for Monitored Parameters

BILE (ml)	PARAMETER	DAY 1	DAY 3	DAY 5	DAY 7
CB@0	pH	7.03±0.01	7.32±0.04	7.29±0.03	7.31±0.01
	D.O. (mg/l)	0.79±0.03	1.36±0.06	1.81±0.12	2.17±0.22
	Temperature (°C)	25.85±0.07	26.85±0.35	26.95±1.06	27.35±0.21
	Microbial Count (CFU/ml)	1.05±0.03E+21			1.38±0.01E+21

CB@10	pH	7.05±0.06	7.15±0.01	7.24±0.03	7.32±0.01
	D.O. (mg/l)	1.07±0.02	1.57±0.02	2.21±0.04	2.67±0.02
	Temperature (°C)	27.00±0.14	28.40±0.14	28.55±1.20	28.45±0.64
	Microbial Count (CFU/ml)	1.04±0.01E+21			1.42±0.01E+21
CB@20	pH	7.02±0.03	7.22±0.01	7.22±0.16	7.39±0.06
	D.O. (mg/l)	1.17±0.01	1.66±0.04	2.29±0.04	2.80±0.02
	Temperature (°C)	28.65±0.78	29.50±1.41	28.70±0.28	28.90±0.14
	Microbial Count (CFU/ml)	1.06±0.02E+21			1.45±0.01E+21
CB@40	pH	7.03±0.04	7.18±0.08	7.21±0.00	7.27±0.04
	D.O. (mg/l)	1.33±0.01	1.88±0.02	2.57±0.03	3.08±0.03
	Temperature (°C)	28.80±0.42	29.80±0.14	28.35±0.78	29.05±0.50
	Microbial Count (CFU/ml)	1.06±0.01E+21			1.49±0.00E+21
GB@10	pH	7.01±0.00	7.23±0.01	7.30±0.01	7.32±0.06
	D.O. (mg/l)	1.04±0.01	1.57±0.05	2.20±0.01	2.62±0.02
	Temperature (°C)	28.10±0.28	28.60±0.85	27.40±0.85	28.50±0.85
	Microbial Count (CFU/ml)	1.04±0.01E+21			1.40±0.01E+21
GB@20	pH	7.06±0.02	7.24±0.04	7.25±0.08	7.36±0.07
	D.O. (mg/l)	1.16±0.01	1.67±0.02	2.18±0.04	2.72±0.03
	Temperature (°C)	28.45±0.21	28.90±0.42	28.95±1.20	29.10±0.57
	Microbial Count (CFU/ml)	1.05±0.01E+21			1.43±0.01E+21
GB@40	pH	7.05±0.02	7.10±0.16	7.17±0.21	7.22±0.18
	D.O. (mg/l)	1.30±0.02	1.84±0.01	2.51±0.04	2.98±0.01
	Temperature (°C)	28.80±0.28	28.85±1.34	28.65±0.21	28.80±0.14
	Microbial Count (CFU/ml)	1.06±0.00E+21			1.49±0.01E+21
CB+GB@10	pH	7.04±0.01	7.26±0.10	7.27±0.02	7.32±0.06
	D.O. (mg/l)	1.14±0.03	1.65±0.01	2.43±0.03	2.90±0.02
	Temperature (°C)	28.25±0.21	28.50±0.99	28.75±0.92	29.30±0.42
	Microbial Count (CFU/ml)	1.07±0.01E+21			1.47±0.02E+21
CB+GB@20	pH	7.03±0.11	7.24±0.02	7.23±0.01	7.35±0.03
	D.O. (mg/l)	1.25±0.01	1.90±0.01	2.55±0.02	3.15±0.04
	Temperature (°C)	27.95±0.78	28.65±0.07	28.95±0.35	28.65±0.07
	Microbial Count (CFU/ml)	1.04±0.01E+21			1.52±0.02E+21
CB+GB@40	pH	7.00±0.01	7.05±0.06	7.23±0.11	7.31±0.03
	D.O. (mg/l)	1.40±0.01	2.20±0.05	2.63±0.03	3.33±0.02
	Temperature (°C)	28.80±0.85	29.60±0.71	29.00±1.56	29.35±0.50
	Microbial Count (CFU/ml)	1.06±0.01E+21			1.58±0.01E+21

Table 5: Results for Nutritional and Microbial Load of Applied Biles

PARAMETER	BILE	
	CB	GB
Na (mg/l)	20.09±0.13	19.18±0.09
Mg (mg/l)	9.88±0.20	11.25±0.21
Ca (mg/l)	2.55±0.07	2.61±0.02
K (mg/l)	7.56±0.14	5.94±0.07
P (mg/l)	6.80±0.03	5.10±0.03
C (%)	3.14±0.23	2.96±0.10
N (%)	4.12±0.05	3.39±0.24
Microbial Count (CFU/ml)	1.36±0.51E+21	1.01±0.73E+21

4. Discussion

Biles are rich sources of minerals [38]. Cow and goat biles as the present study confirmed contained appreciable quantities of the minerals N, P, K, Na, Ca, Mg and C.

The study in general recorded higher increases in microbial mass and TPH removal at higher bile levels [Table 1]. The observation is suggestive of the adaptive response of hydrocarbon-eating microbes to the presence of the hydrocarbons (applied oil) and available nutrients (applied biles). Thus increases in microbial numbers and the corresponding TPH removal rates across all levels of bile application are indicative of the utilization of the applied oil by hydrocarbon-eating microbes as carbon source and the utilization of the applied biles as nutrient sources [15]. The above observation could also be explained in relation to the fact that applied biles were microbially enriched on characterization and as such there exists the possibility that significant numbers of microbes with the ability to degrade hydrocarbons were introduced in quantities proportional to the applied levels. These microbes thus typically augmented the activities of hydrocarbon-eating microbes originally introduced to undertake the breakdown of the applied oil.

Bile to bile performances at each tested level showed different trends for both microbial mass increments and TPH removal. The respective trends were CB+GB>CB>GB and CB+GB>GB>CB. The observed microbial mass increment trend could be explained in relation to the heterotrophic microbial load of applied biles. CB was found to have a higher heterotrophic microbial load than GB [Table 5]. This however did not translate into a corresponding TPH removal trend as GB ended up triggering a higher TPH removal than CB. Hydrocarbon-eating microbes within the heterotrophic pool of CB possibly suffered a delay in getting acclimatized to pertaining conditions within the reactor setup and the presence of the applied oil. CB+GB emerged the best performing bile in terms of its impacts on microbial mass increase and TPH removal. This was anticipated as CB+GB offered a richer pool or cocktail of essential nutrients than the individual biles. CB+GB in addition also served as a cocktail of diverse microorganisms that probably had the capacity for hydrocarbon degradation. Similarly, applied level performances across each tested bile assumed the trend 40>20>10 for microbial mass increases. For TPH removal rates, same performance trend as realized for microbial mass increase was realized for CB and CB+GB while for GB the performance pattern was 20>40>10. It can be inferred from the GB trend that microbes at applied level of 40 ml may have suffered some level of inhibition that possibly caused a delay/time lapse in microbial acclimatization to pertaining conditions within the reactor setup and the presence of the applied oil.

Higher bile levels typically introduced appreciable quantities of C on application taking into account the fact that applied biles were as well rich in organic C. Hydrocarbon-eating microbes were thus presented with a dual carbon source for utilization, a situation that was expected to impede the rate of uptake and breakdown of hydrocarbons. However, relatively high rates of TPH removal were recorded at all levels of bile application suggestive of the preference of hydrocarbon-eating microbes for the applied oil as the primary carbon source; an observation that conforms to that of [39].

Though animal bile has been cited as one class of naturally occurring compounds with surfactant attributes that can potentially render performances comparable to microbially induced surfactants [40], hydrocarbon remediation studies involving the use of animal bile as surfactant are sparse. Per the present study, cow and goat biles contributed to the observed increases in microbial mass and TPH removal rates via their role as biosurfactants. Cow and goat biles as applied in the present study typically augmented biosurfactants that may have been naturally produced by hydrocarbon-eating microbes in response to the presence of the hydrocarbon contaminant. Thus, application of the aforementioned biles typically may have facilitated emulsification and spread/dispersal of the hydrocarbon contaminant within the water column. Observed corresponding increases in microbial numbers and TPH removal rates at the various applied bile levels were found to be consistent with increasing dissolved oxygen (D.O) levels recorded.

Appreciable hydrocarbon degradation rates (>80 %) as achieved in the present study occurred over respective optimum pH, D.O and temperature ranges of 7.00±0.01 to 7.39±0.06, 0.79±0.03 to 3.33±0.02 mg/l, 25.85±0.07 to 29.80±0.14 ^{o}C. These ranges are consistent with that reported in a similar study by [5]. Per the recorded pH range, hydrocarbon degraders were chiefly of bacteria genera. As cited by [15], hydrocarbon degraders of bacteria genera are more inclined to near neutral pH conditions with the fungal genera more inclined to acidic conditions. Higher rates of hydrocarbon degradation have been associated with near neutral pH conditions predominantly slightly alkaline pH [41-44]. The relatively high TPH removal rates attained in the present study confirms the preference of hydrocarbon-eating microbes for near neutral pH settings. The slightly alkaline conditions observed in

the present study invariably may be indicative of the production of alkaline intermediates.

Also established in the present study was a correspondence between increasing D.O levels and high TPH removal rates. The observed correspondence essentially suggest active utilization of supplied oxygen by aerobic hydrocarbon-eating microbial consortia. Increasing D.O. levels can be inferred to be indirectly indicative of the rate of emulsification/breakdown of applied oil by aerobic hydrocarbon-eating microbes.

Again, per the recorded temperature range, psychrotrophic and mesophilic bacteria to a larger extent were at play in the degradation of hydrocarbons in the present study. According to [45] optimum hydrocarbon degradation typically occurs in the range of 20 to 35 °C. This is consistent with the range of 20 to 30 °C reported by [46]. Recorded temperature range for hydrocarbon degradation in the present study was thus consistent with the findings of the abovementioned researchers. The temperature range recorded in the present study may have been influenced by the ambient temperature to which the setup was subjected. Ambient temperature aside its influence in defining hydrocarbon-eating microbial communities may have facilitated hydrocarbon losses via volatilization/evaporation [15, 41, 47].

Applied support media achieved microbial fixation via adsorption. This typically facilitated direct contact between hydrocarbon-eating microbes and applied oil (petroleum) as well as available nutrients within the medium. Adsorption of hydrocarbon-consuming microbes to support media surfaces was expected taking into account the inherent ability of microbes to adhere to surfaces to form biofilms as has been established in studies [2, 48]. The present study similarly established significant microbial numbers on the surfaces of applied support media [Table 4].

5. Conclusions and Recommendations

Cow and goat biles and a 1:1 mix of both biles were successfully applied as amendments for hydrocarbon-eating microbes towards hydrocarbon degradation in water. Applied bile amendments triggered appreciable increases in applied microbial numbers which translated into appreciable TPH removals. Significant interaction effect existed between hydrocarbon-eating microbes (attached to support media surfaces) and the applied bile amendments to ensure the survival of hydrocarbon-eating microbes towards hydrocarbon degradation ($p<0.05$). The technique proved efficient in treating hydrocarbon contaminated water appreciably.

Contamination issues associated with the use of petroleum are inevitable and should be anticipated. The anticipation of such occurrences calls for search into viable remediation options that are also environmentally friendly. The present study is one such remediation option that has shown capability of treating hydrocarbon contaminated water to appreciable levels. The technology presents the petrochemical industry in Ghana and elsewhere with an environmentally friendly and viable technological option for exploitation in the treatment of petrochemical effluents prior to discharge. It also presents an option as a pump and treat technology for the remediation of surface water and ground water.

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