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ORIGINAL RESEARCH PAPER

Hydrocarbon contaminated water remediation using a locally constructed multi-stage bioreactor incorporated with media filtration

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ABSTRACT: The present study investigated the coupling effect of biodegradation and media filtration in treating hydrocarbon contaminated water. The study recorded reductions in total petroleum hydrocarbon, total dissolved solids, turbidity and microbial load. The study was essentially a simulated pump and treat process that involved the pumping of hydrocarbon contaminated water for treatment in a locally designed multi-stage bioreactor incorporated with media filtration. A mixed consortium of hydrocarbon-eating microbes was applied in the study. Hydrocarbon-eating microbes were isolated from hydrocarbon contaminated soils obtained from selected mechanic workshops. Bamboo chips and coconut husk chips were applied as support media for microbial attachment within the bioreactor compartment of the treatment setup. Applied support media were approximately 2-4 cm in size. Media filters applied comprised three locally manufactured candle filters two of which were respectively impregnated with granular activated charcoal and sand. The coupling effect of biodegradation and media filtration recorded over 99 % (> 8.7 mg/L) total petroleum hydrocarbon removal. Microbial load reduction ranged from 3.57±0.11E+20 to 7.45±0.26E+20 Colony forming unit/mL, total dissolved solids reduction from 30.00±5.66 to 131.00±0.00 mg/L and turbidity reduction from 39.00±1.41 to 123.50±0.71 nephelometric turbidity units. Biodegradation accounted for 69.70±0.63 and 90.72±2.36 % total petroleum hydrocarbon removal respectively for bamboo chips and coconut husk chips.

Keywords: *Biodegradation; Bioremediation; Fixed-bed bioreactor; Hydrocarbon contamination; Media filtration; Support media.*

INTRODUCTION

The reliance on petroleum as an energy source the world over makes hydrocarbon contamination a global issue of concern (Bayat *et al.,* 2015; Das and Chandran, 2010; Diyauddeen *et al.,* 2011; Mandri and Lin, 2007). Petroleum is the most consumed of all primary energy sources. It accounts for about 36 % of the hydrocarbon fuel energy sources (Corbo *et al.,* 2011). Routes of entry of petroleum into the environment are often via accidental spills and leaks during exploration, production, transportation, usage and storage of petroleum or petroleum products (Abioye *et al*., 2012; Das and Chandran, 2010; National Research Council, 1985). Also implicated is the indiscriminate discharge of hydrocarbon rich wastes such as used motor oil and petrochemical effluents into the environment. Thus accidental

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spills and leaks coupled with unscrupulous disposal practices constitute the main routes of increased loading of hydrocarbons into both soil and aquatic environments (Abioye *et al*., 2012; Antwi-Akomeah *et al*., 2018a). Petroleum contamination via the abovementioned routes reaches several million tons annually on a global scale, a phenomenon that is chronic (Das and Chandran, 2010). The presence of hydrocarbon contaminants in aquatic and soil media invariably constitute public and socio-economic threats to living organisms taking into account their contact toxicity, carcinogenic and neurotoxic attributes (Adelowo *et al*., 2006; Das and Chandran, 2010). A number of physico-chemical treatment options have received application in the remediation of hydrocarbon contaminants in both aquatic and soil media. These include incineration, chemical extraction, and burial in secure landfills among others. Incomplete contaminant breakdown/removal and the production of toxic intermediates/products have often characterized physico-chemical treatment options. Bioremediation on the other hand has received favourable consideration over physico-chemical treatment techniques owing to its ability to render complete mineralization and removal of organic contaminants and the fact that it is environmentally friendly and non-invasive (Abdul Rahim and Gaber, 2010; Varjani and Upasani, 2017). Bioremediation essentially exploits the catalytic or enzymatic attributes of adapted microbes to convert target contaminants such as hydrocarbons into less toxic or non-toxic products such as water, carbon dioxide, biomass, etc. (Adams *et al*., 2015; Das and Chandran, 2010; Joutey *et al*., 2013; Varjani and Upasani, 2017). Citing Ghana as case study, the commercial production of crude oil since 2010 has not been without issues of petroleum contamination. A year into the commercial production of crude oil, there were reports of minor oil spills along the coasts of Ahanta West and along the beaches of Jomoro and Ellembelle in the Western Region of Ghana. Aside the minor spills reported so far, there is the daily generation of hydrocarbon rich wastes chiefly used motor oil and petrochemical effluents from mechanic workshops, automobile filling stations and other petrochemical establishments such as the Tema Oil refinery (Fei-Baffoe *et al*., 2012). In Ghana and elsewhere, huge volumes of these hydrocarbon rich wastes are carelessly discharged into the environment (soil and water bodies) without treatment. With reference to the manner and rate at which petroleum/petrochemical establishments are springing up in Ghana and elsewhere, the study is a necessity. It is a potentially applicable mitigation technology in the wake of petroleum contamination of surface and ground waters. The current technology presents the petroleum industry and related establishments in Ghana and elsewhere with a relatively cheap and viable remediation option for the treatment of petrochemical effluents prior to discharge as well as dealing with surface and ground water contamination that may result not only from hydrocarbons but also similar recalcitrant compounds. The present study sought to 1) evaluate the performance of bamboo chips (BC) and coconut husk chips (CHC) as biofilm support media (BSM) for microbial fixation towards hydrocarbon degradation; 2) evaluate the performance of locally designed media filters in reducing total petroleum hydrocarbon (TPH) levels, total dissolved solids (TDS), turbidity and microbial load of the treated water; 3) evaluate the synergized impact of biodegradation and media filtration on TPH removal. The study was performed at the Department of Theoretical and Applied Biology of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in 2018.

MATERIALS AND METHODS

Design and operation of treatment setup

The treatment setup comprised a total of eight reactors and three media filters configured as shown in Fig. 1. Each reactor comprised a vessel and column. The volume of each vessel and column was approximately 0.0091 and 0.0024 m^3 respectively. The first three pairs of reactors were aerated using an aquarium air pump. The last pair was left anaerobic. The treatment setup operated on the pump and treat principle. The setup operated on three 0.5 hp water pumps each coupled with an electronic timing device. Six cycling regimes were applied per day in each experiment. Each cycling regime lasted for 10 min. Sample concentrate was pumped from storage tank 1 through the reactors into storage tank 2 at a flow rate of 0.5 L/min. Sample flow from storage tank 2 into storage tank 1 was performed at a flow rate of 2 L/min. every 24 hours (h.). Flow of bio-treated sample from storage tank 2 into the media filters was performed at a flow rate of 2 L/min. after 7 days of cycling through the bioreactor compartment. Each

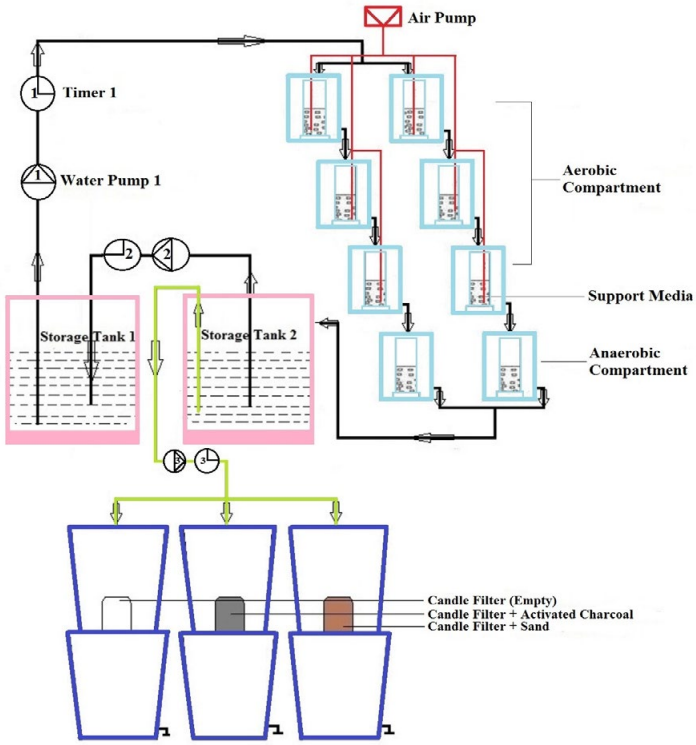

Fig. 1: Configuration of remediation setup

incorporated filtration setup comprised a transparent 10 L plastic bucket fitted with a candle filter. Candle filters were made from kaolin clay and local Mfensi clay. Two of the candle filters were impregnated with granular activated charcoal (ACF) and sand (SCF) respectively (Fig. 1); (Antwi-Akomeah *et al*., 2018a, 2018b).

Pre-treatment of reactor vessels, columns and candle filters

Reactor vessels and columns prior to their application were thoroughly washed with soap and water. They were subsequently rinsed with 10 % NaOCl solution followed by 70 % ethanol solution and finally with sterile distilled water (Antwi-Akomeah *et al*., 2018a, 2018b; Fukuzaki, 2006; Rutala *et al*., 2008). Candle filters were also pre-treated by initially immersing them in 10 % NaOCl solution followed by immersion in 70 % ethanol solution (Fukuzaki, 2006; Rutala *et al*., 2008) and finally in sterile distilled water. Immersion in each instance lasted for 10 min.

Biofilm support media and pre-treatment

BC and CHC were applied as biofilm support media (BSM) for microbial attachment. BC and CHC were respectively fabricated from fresh bamboo canes and coconut husks. Fresh bamboo canes and coconut husks were chopped into pieces approximately 2-4 cm in size. BC and CHC were thoroughly washed with soap and water, air dried and subjected to dry heat sterilization in hot air oven at 180 ^{o}C for 30 min. (Darmady *et al*., 1961; Rutala *et al*., 2008). The respective masses of BC and CHC applied were 0.71 kg and 0.67 kg per reactor. Each support media was applied for a week with replication.

Activated charcoal and sand media pre-treatment

Activated charcoal and sand were subjected to dry heat sterilization in hot air oven at a temperature of 180 ^{o}C for 60 min. (Fukuzaki, 2006; Rutala *et al*., 2008) and allowed to cool to room temperature before application. ACF was impregnated with 150 g of sterile activated charcoal while SCF was filled with 150 g of sterile sand.

Cultivation of hydrocarbon eating microbes

Hydrocarbon-eating microbes were isolated from hydrocarbon contaminated soil obtained from identified mechanic workshops. Three mechanic workshops were sampled. Soil samples were fetched into sterile petri dishes and transported to the laboratory where they were homogenized. 10 g of homogenized hydrocarbon contaminated soil was weighed into a 200 mL beaker and 50 mL of distilled water added. The mixture was stirred for 3 min. using a magnetic stirrer. 1 mL of the resulting solution was drawn and pour plated on mineral salt-agar medium (Fei-Baffoe *et al*., 2012). About 0.25 mL of crude oil was spread on the mineral salt-agar medium upon setting and left to stand for an h. The plate was incubated at 37 ^{o}C for 72 h. (Okpokwasili and Amanchukwu, 1988). Growths appearing after the incubation period were washed with sufficient amount of mineral salt solution into a 1000 mL Erlenmeyer flask. Additional mineral salt solution was added to make the mark. Liquid culture was inoculated with 2 drops of crude oil and incubated at 37 ^{o}C for a further 48 h. (Fei-Baffoe *et al*., 2012).

Characterization of hydrocarbon-eating bacteria

Microbial differentiation was by Grams technique. A microbial smear was prepared using an oil free slide. The slide was initially cleaned with chromic acid. The slide was subsequently washed with water and stored in alcohol for couple of min. It was removed and passed through a Bunsen flame to remove alcohol. A drop of prepared liquid culture was placed on the slide and a sterile loop applied in spreading the drop across the face of the slide. The slide was left to dry and then passed through a Bunsen flame a couple of times to fix microbes. The smear was stained initially with 0.5 % crystal violet for 1-2 min. washed with water and allowed to drain. The smear was then stained with Lugol's iodine for 1-2 min. and washed off with water. It was then washed with ethanol for few seconds followed by water. It was then counterstained with 1 % safranin for 2 min. washed with clean water and allowed to air dry. The slide was then subjected to microscopy. The 40X objective was used in locating the stained smear. A drop of immersion oil was then placed on the smear and examined with the oil immersion (100X) lens (Cheesbrough, 2006).

Preparation of mineral salt-agar medium

Mineral salt-agar medium was prepared by combining the recipes of Kastner *et al*. (1995), Mills *et al*. (1978) and Zajic and Supplisson (1972). Mineral salt solution containing the salts- sodium chloride (NaCl), sodium phosphate dibasic dihydrate ($Na_2HPO_4.2H_2O$), potassium dihydrogen phosphate (KH_2PO_4), ammonium chloride (NH_4Cl), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) and iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$) was prepared. Respective quantities of 8.0 g, 2.5 g, 1.5 g, 1.0 g, 0.5 g and 0.05 g of the above salts were weighed into a 1000 mL Erlenmeyer flask and distilled water added to make the mark. The mineral salt solution was stirred to facilitate dissolution of the salts. 20 g of purified agar was subsequently suspended in the prepared mineral salt solution and heated on a hot plate to hasten dissolution of the agar. The mixture was autoclaved for 15 min. at 121 ^{o}C and allowed to cool appreciably prior to use.

Conditioning of hydrocarbon degrading microbes

Hydrocarbon-degrading microbes prior to each experiment were introduced onto support media packed inside each column of a reactor. 125 mL of prepared liquid culture was poured onto the support media inside each column and allowed to stand for 48 h. with gentle manual agitations at regular intervals. Liquid culture was poured off after the 48 h. period (Antwi-Akomeah *et al*., 2018a, 2018b; Fei-Baffoe *et al*., 2012).

Hydrocarbon source and concentration tested

Applied crude oil was obtained from Ghana's Jubilee Oil Field located 60 km offshore between Deepwater Tano and West Cape Three Points blocks off the coast of the Western Region of Ghana (REIMG, 2013). A sample concentration of 1000 mg/L was applied in each experimental run.

Hydrocarbon eating and total heterotrophic microbial enumeration

Hydrocarbon-eating microbes were enumerated by plate count employing serial dilution and pour plating techniques. Hydrocarbon-eating microbes were pour-plated on mineral salt-agar medium (Chikere *et al*., 2009; Chimezie Dirisu, 2015). Microbes adhering to the surfaces of support media were eluted into 50 mL of distilled water. 1 mL of

the sample was drawn and diluted in tenfold through to 10^{-19}. Dilutions 10^{-16}, 10^{-17}, 10^{-18} and 10^{-19} were plated out in duplicates by the pouring technique. A sample volume of 1 mL was plated in each instance. Plates were supplemented with about 0.25 mL crude oil (by spreading on the surface of the media) and left to stand for an h. Plates were incubated at 37 °C for 72 h. Petri-dishes prior to their application were heat sterilized in hot air oven for 2 h. at 180 °C and allowed to attain room temperature prior to use. Total heterotrophic microbes were similarly enumerated by serial dilution and pour plating on nutrient agar medium. 1 mL of sample was diluted serially and plated as described above. Plates were incubated at 37 °C for 48 h. The average microbial colonies per mL of plated dilutions were estimated using Eq. 1 (Antwi-Akomeah *et al*., 2018a, 2018b; Fei-Baffoe *et al*., 2012; Goldman and Green, 2008; Kilduff *et al*., 2000).

Colony forming unit (CFU/mL) = (Number of colonies x Dilution factor)/Volume plated (1)

Determination of total petroleum hydrocarbons (TPH)

TPH was determined by gas chromatography coupled with a flame ionization detector (GC-FID) under the following conditions:

Carrier gas flow rate	5 mL/min.
Initial temperature	40 °C, hold for 0.5 min.
Program	40 °C to 290 °C at 15 °C/min.
Final temperature for 10 min.	290 °C, hold
Injector temperature	290 °C
Detector temperature	300 °C
Make-up gas	25 mL/min.

An alkane standard sample of concentration 1 mg/L was run through the instrument under the above stated conditions. The standard sample peak retention time and area were then compared to that of the test samples to calculate their concentrations. C_9-C_{36} range of hydrocarbons was measurable with this method. GC-FID analysis was preceded by TPH extraction from samples (Antwi-Akomeah *et al*., 2018a, 2018b; Environmental Research Institute, 1999; Fei-Baffoe *et al*., 2012).

TPH extraction

500 mL of sample was transferred into a 1000 mL separatory funnel and 50 mL of methylene chloride added. The separatory funnel with its content was briefly shaken and left to stand for 15 min. The organic fraction was carefully drained into a 100 mL beaker. Extraction was repeated two more times with 25 mL methylene chloride in each instance. Methylene chloride and water traces were removed from the organic extract using the Soxhlet extractor. The final sample extract was concentrated in 2 mL of methylene chloride and subsequently transferred into a 2 mL vial to obtain the test sample (Adebusoye *et al*., 2006; Antwi-Akomeah *et al*., 2018a, 2018b; Fei-Baffoe *et al*., 2012).

Temperature, pH, D.O, and TDS determination

Temperature, pH, D.O, and TDS were determined using a multi-parameter reading probe (YSI 600XL) and meter (YSI 650 MDS). The probe was calibrated prior to its use following manufacturer's instructions. The probe was rinsed with distilled before and after each sample reading. Probe was dipped into sample, held in place for a couple of min. until a stable reading was attained and recorded (Antwi-Akomeah *et al*., 2018a, 2018b; Rahmanian *et al*., 2015). Temperature, pH and D.O were determined at 48 h. intervals. TDS was determined prior to and after filtration.

Turbidity measurement

Turbidity of the bio-treated water prior to and after filtration in nephelometric turbidity units (NTU) was measured using the 2100Q Portable Turbidimeter. Turbidimeter was calibrated prior to its use with a formazin primary standard of known turbidity (4000 NTU). Sample was transferred into the cuvette and placed inside the cuvette holder for a couple of min. until a stable reading was attained (Rahmanian *et al*., 2015).

Statistical analysis

Results from the study were statistically analyzed using SPSS 20 and Microsoft Office Excel 2007 software packages. One way multivariate analysis of variance was performed using SPSS 20 software package to test the interaction effect between BSM and applied microbes as well as the effect of BSM on MI and TPH removal. Pairwise comparisons among

filter performances were determined by Tukey's test using SPSS 20 software package. Bar charts were drawn using Microsoft Office Excel 2007 software package.

RESULTS AND DISCUSSION

Biofilm support media (BSM) impact

BSM performance was evaluated by comparing the performances of BC and CHC in each instance with a blank run (BR) in which the applied microbes were freely suspended. BR was thus devoid of any BSM. Freely suspended microbes increased by an amount of 1.90±0.00E+20 CFU/mL. TPH removal by freely suspended microbes was 5.531±0.196 mg/L. The application of BC and CHC as support media for microbial fixation saw respective increments of 3.50±0.14E+20 CFU/mL and 4.00±0.14E+20 CFU/mL in microbial numbers. TPH removal by microbes fixed unto support media were respectively 6.191±0.056 mg/L and 8.057±0.210 mg/L for BC and CHC. BC and CHC performances were respectively 14.60±0.00 % and 20.40±1.27 % higher than BR with regards to the recorded microbial increments. In terms of TPH removal, BC and CHC performances were respectively 7.40±1.59 % and 28.43±0.14 % higher than BR (Table 1). Recorded increments in microbial load (MI) and TPH removal were in each instance highest for CHC support media.

Figs. 2a and 2b respectively, denote BSM impact on microbial growth and TPH removal.

BSM essentially provided surfaces for microbial attachment within the bioreactor compartment. BSM as established by the present study recorded increments in both microbial numbers and TPH removal (Table 1). Recorded increments in microbial numbers and TPH removal could be ascribed to the adsorption or adhesion of microbial cells unto support media which in principle would facilitate direct contact between microbes and hydrocarbons as well as available nutrients within the medium (Braschler *et al*., 2015; Martins *et al*., 2013). Adsorption/attachment of hydrocarbon-eating microbes to support media was imminent owing to the propensity of microbes to adhere to surfaces via weak interactive forces such as van der Waal forces, ionic and hydrogen bonding etc. to form biofilms

Table 1: Results for BSM impact

BSM (kg)	TPH removal		Microbial load increment		Performance	
	mg/L	%	CFU/mL	%	TPH (%)	MI (%)
BR	5.531±0.196	62.29±2.22	1.90±0.00E+20	18.25±0.21	0.00±0.00	0.00±0.00
BC	6.191±0.056	69.70±0.63	3.50±0.14E+20	32.85±0.21	7.40±1.59	14.60±0.00
CHC	8.057±0.210	90.72±2.36	4.00±0.14E+20	38.65±1.06	28.43±0.14	20.40±1.27

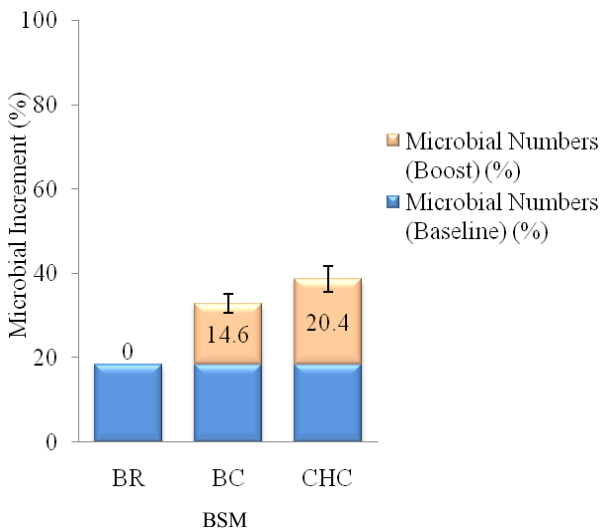

Fig. 2a: BSM impact (Microbial growth)

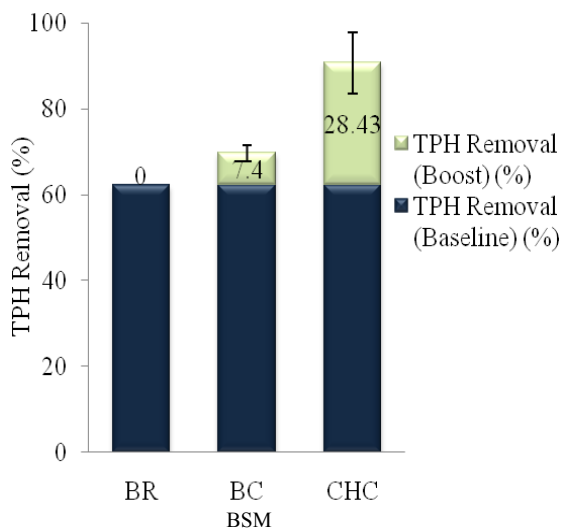

Fig. 2b: BSM impact (TPH removal)

(Bayat *et al*., 2015; Górecka and Jastrzębska, 2011; Martins *et al*., 2013). CHC emerged the better performer of the two support media (CHC > BC). BSM performance could also be explained in relation to the nature of the applied support media. Support media nature would determine in part the surface area available for colonization by microbes. CHC by virtue of its spongy/fibrous nature typically offered a relatively larger surface area for microbial attachment in comparison to BC (Dong-Hao *et al*., 2013; Górecka and Jastrzębska, 2011). With regards to TPH removal, microbes immobilized unto BC and CHC support media achieved removal rates that were respectively 7.40±1.59 % and 28.43±0.14 % higher than BR. This performance is consistent and comparable to that of similar plant materials including Loofah sponge, sugarcane bagasse, corn cob, etc. that have received applications in similar studies (Dzionek *et al*., 2016). 79 % degradation of hydrocarbons by hydrocarbon-eating microbes attached onto Loofah sponge was reported by Maliji *et al*. (2013). This performance was 5 % more than the performance of the same microbes when freely suspended. Similarly, the use of sugarcane bagasse for microbial immobilization resulted in 76.8 % degradation of hydrocarbons whiles same microbes upon suspension only degraded 22.3 % of the hydrocarbons (Lin *et al*., 2015). 56 % and 33 % degradation of hexadecane was also reported by Rivelli *et al*. (2013) respectively for microbes immobilized onto corn cob and when freely suspended. Antwi-Akomeah *et al*. (2018a) also reported of about 70.2 % TPH removal using bamboo chips as support media. TPH removals recorded in the present study were achieved in a relatively shorter timescale of 7 days compared to similar studies such as that reported by Lin *et al*. (2015), Maliji *et al*. (2013), Rivelli *et al*. (2013), among others. Multivariate main effect for BSM was significant per one way multivariate analysis of variance (MANOVA) conducted- Wilks' $\lambda = 0.000$, $F(2, 4) = 68.721$, $p = 0.014$, partial eta squared = 0.993. Power to detect the effect was 1.000. Univariate main effect of BSM was significant for TPH, $F(2, 2) = 102.127$, $p = 0.010$, partial eta square = 0.990, power = 0.994. Univariate main effect was also significant for microbial growth increases (MI), $F(2, 2) = 407.251$, $p = 0.002$, partial eta square = 0.998, power = 1.000. Significant interaction ($p < 0.05$) between hydrocarbon-eating microbes and support media was evident statistically.

Coupling effect of biodegradation and media filtration

Over 99 % TPH removal was achieved for the synergized impact of biodegradation and media filtration (Table 2). Media filtration was performed after 7 days of bio-treatment in the bioreactor compartment of the treatment setup. Media filtration following bio-treatment with freely suspended microbes (BR study) achieved TPH removals of 3.26±0.19 mg/L, 3.30±0.18 mg/L and 3.33±0.20 mg/L respectively for CF, SCF and ACF. Filtration following bio-treatment with BC-immobilized microbes accounted for TPH removals of 2.64±0.05 mg/L, 2.68±0.06 mg/L and 2.64±0.06 mg/L respectively for CF, SCF and ACF. Similarly, filtration after bio-treatment with CHC-immobilized microbes ensured TPH removals 0.80±0.21 mg/L, 0.81±0.21 mg/L and 0.81±0.21 mg/L respectively for CF, SCF and ACF (Table 3). The above TPH removal performances denote filter efficiencies of 96.69±0.76 to 97.92±0.11 %, 97.77±0.40 to 99.40±0.17 % and

Table 2: Results for coupling effect of biodegradation and filtration

Treatment	Initial TPH	Final TPH	TPH removal	
	mg/L	mg/L	mg/L	%
$CF_{BR+BIOTREATMENT}$	8.882±0.00	0.088±0.005	8.795±0.005	99.02±0.05
$SCF_{BR+BIOTREATMENT}$	8.882±0.00	0.050±0.016	8.832±0.016	99.44±0.18
$ACF_{BR+BIOTREATMENT}$	8.882±0.00	0.025±0.002	8.858±0.002	99.73±0.02
$CF_{BC+BIOTREATMENT}$	8.882±0.00	0.056±0.004	8.826±0.004	99.37±0.04
$SCF_{BC+BIOTREATMENT}$	8.882±0.00	0.016±0.004	8.866±0.004	99.82±0.04
$ACF_{BC+BIOTREATMENT}$	8.882±0.00	0.048±0.001	8.834±0.001	99.46±0.01
$CF_{CHC+BIOTREATMENT}$	8.882±0.00	0.027±0.000	8.856±0.001	99.71±0.01
$SCF_{CHC+BIOTREATMENT}$	8.882±0.00	0.018±0.001	8.864±0.001	99.80±0.01
$ACF_{CHC+BIOTREATMENT}$	8.882±0.00	0.015±0.003	8.867±0.003	99.83±0.03

Table 3: Results for media filtration

Filter	TPH removal		MR		TDS reduction		TR	
	mg/L	%	CFU/mL	%	mg/L	%	NTU	%
CF_{BR}	3.26±0.19	36.73±2.17	3.57±0.11E+20	29.04±1.26	30.00±5.66	22.04±2.22	39.00±1.41	27.41±1.94
SCF_{BR}	3.30±0.18	37.15±2.04	5.09±0.14E+20	41.38±0.69	49.00±8.49	36.03±3.06	66.00±0.00	46.35±1.61
ACF_{BR}	3.33±0.20	37.44±2.24	6.03±0.08E+20	49.01±0.08	68.00±4.24	50.25±1.32	78.50±2.12	55.10±0.43
CF_{BC}	2.64±0.05	29.68±0.59	4.29±0.22E+20	30.28±2.15	56.50±2.12	23.01±0.27	61.00±1.41	24.43±1.39
SCF_{BC}	2.68±0.06	30.13±0.67	5.91±0.61E+20	41.71±2.45	98.00±4.24	39.91±0.69	109.50±3.54	43.80±0.07
ACF_{BC}	2.64±0.06	29.77±0.64	7.08±0.40E+20	49.98±0.63	119.50±3.54	48.68±0.18	123.50±0.71	49.44±1.96
CF_{CHC}	0.80±0.21	8.99±2.35	4.48±0.22E+20	31.18±1.07	78.50±7.78	30.23±2.42	41.50±0.71	23.24±0.83
SCF_{CHC}	0.81±0.21	9.08±2.35	6.18±0.23E+20	43.06±0.96	90.00±2.83	42.00±2.34	75.00±5.66	36.39±2.45
ACF_{CHC}	0.81±0.21	9.11±2.33	7.45±0.26E+20	51.89±1.02	131.00±0.00	50.49±0.96	86.50±3.54	48.45±1.03

Table 4: Results for filter efficiency

Filter	Initial TPH	Final TPH	TPH removal	
	mg/L	mg/L	mg/L	%
CF_{BR}	3.349±0.197	0.0875±0.005	3.26±0.19	97.39±0.007
SCF_{BR}	3.349±0.197	0.0500±0.016	3.30±0.18	98.52±0.382
ACF_{BR}	3.349±0.197	0.0245±0.002	3.33±0.20	99.27±0.106
CF_{BC}	2.692±0.056	0.0560±0.004	2.64±0.05	97.92±0.113
SCF_{BC}	2.692±0.056	0.0160±0.004	2.68±0.06	99.40±0.170
ACF_{BC}	2.692±0.056	0.0480±0.001	2.64±0.06	98.22±0.092
CF_{CHC}	0.825±0.210	0.0265±0.001	0.80±0.21	96.69±0.764
SCF_{CHC}	0.825±0.210	0.0180±0.001	0.81±0.21	97.77±0.396
ACF_{CHC}	0.825±0.210	0.0150±0.003	0.81±0.21	98.17±0.120

98.17±0.12 to 99.27±0.11 % respectively for CF, SCF and ACF (Table 4). Reductions in microbial load, TDS and turbidity were also recorded. Higher reductions in microbial load, TDS and turbidity were recorded for the impregnated filters (ACF and SCF) compared to CF. Microbial load reduction (MR) ranged from 3.57±0.11E+20 to 7.45±0.26E+20 CFU/mL. TDS reduction ranged from 30.00±5.66 to 131.00±0.00 mg/L with reduction in turbidity (TR) ranging from 39.00±1.41 to 123.50±0.71 NTU (Table 3).

Filtration impact on TPH, microbial load, TDS and turbidity reductions are respectively represented by Figs. 3a, 3b, 3c and 3d.

TPH removal in general terms was marginally higher for ACF and SCF compared to CF. These marginal variations are suggestive of a great deal of hydrocarbon contaminant removal across the CF barrier of ACF and SCF. Recorded reductions in microbial load, TDS and turbidity on the other hand were significantly higher for ACF and SCF compared to CF (Table 3). For each of the above parameters, filter performances were of the order ACF > SCF > CF. The observed trend could be explained in relation to the additional adsorptive barrier conferred by the incorporated activated charcoal and sand media respectively for ACF and SCF. ACF recorded the highest performances in TPH, microbial load, TDS and turbidity removals (Table 3). This observation perhaps is due to the presence of more active sites for contaminant capture owing to the micro-porous nature and oxygen surface groups of activated charcoal (Rodriguez-Reinoso *et al*., 1992). The current study showed consistency with the performance of ceramic filters (Adeyemo *et al*., 2017; Bello *et al*., 2013; Gupta *et al.,* 2016; Shafiquzzaman *et al*., 2011; Subriyer, 2013; Zakaria *et al.,* 2009) and granular filter media (such as sand and activated charcoal) (Adams *et al*., 2017; Ayotamuno *et al*., 2006; Okiel *et al*., 2011) in the treatment of household water and wastewater. As high as 96 % TPH removal has been reported with the use of granular activated carbon as filter media (Ayotamuno *et al*., 2006). Complete removal of hydrocarbons (including cyclopentenone, isocyanoto-methane and ethylcyclopropanol) using a combined filter media of sand + activated charcoal + powdered corn cob has also been reported by Adams *et al*. (2017). Same study recorded 45 % and 23 %

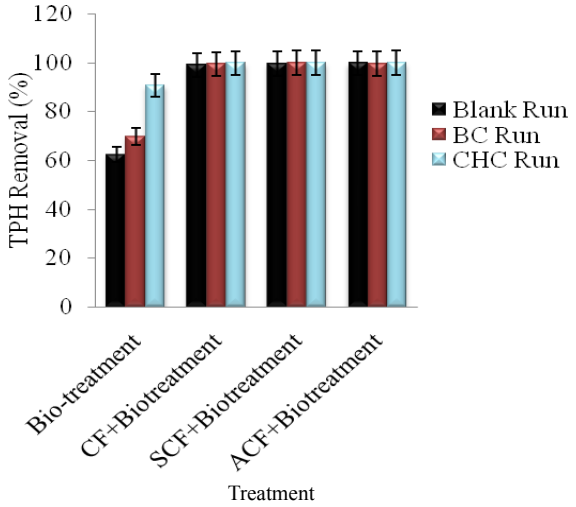

Fig. 3a: TPH removal

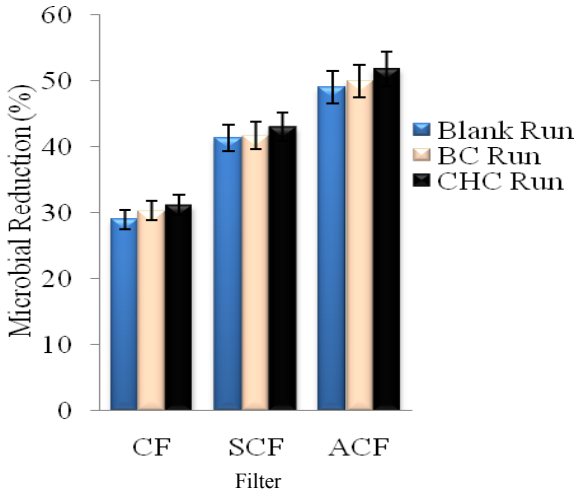

Fig. 3b: Microbial load reduction (MR)

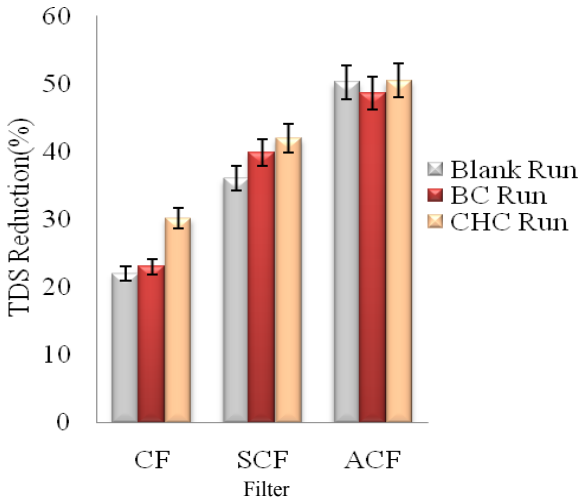

Fig. 3c: TDS reduction

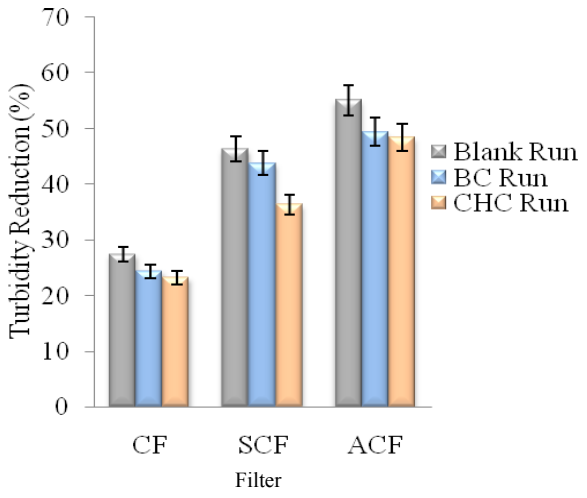

Fig. 3d: Turbidity reduction (TR)

reduction in turbidity, 73 % and 66 % reduction in total dissolved solids (TDS) respectively for individual sand and activated charcoal filters. Several other studies have demonstrated consistency with the findings of Adams *et al*. (2017) and Ayotamuno *et al*. (2006) including the present study. Ceramic candle filters have also proven effective in removing pathogens and other contaminants from drinking water and wastewater (Rahman *et al*., 2015). Between 97.8 % and 100 % removal of *Escherichia coli* from domestic water using ceramic filter submerged in colloidal silver has been reported by Oyanedel-Craver and Smith (2008). In the above cited studies, the adsorptive attributes of the applied filter media were implicated for their ability to reduce contaminants levels appreciably. The adsorptive attributes of applied filter media in the current study can thus also be implicated to have significantly contributed to the attained performances. One way MANOVA showed a significant multivariate main effect for media filtration, Wilks' $\lambda = 0.000$, $F\ (32,\ 23.722) = 13.474$, $p = 0.000$, partial eta

Table 5: Results for monitored parameters (pH, DO, Temperature, Microbial count)

BSM (kg)	Parameter	Day 1	Day 3	Day 5	Day 7
BR	pH	7.04±0.07	7.08±0.09	7.21±0.07	7.28±0.04
	DO (mg/L)	0.80±0.01	1.29±0.03	1.71±0.06	2.15±0.24
	Temperature (°C)	27.50±0.27	27.30±0.53	28.13±0.80	28.40±1.02
	Microbial Count (CFU/mL)	1.04±0.01E+21			1.22±0.02E+21
BC	pH	7.06±0.05	7.32±0.03	7.33±0.08	7.30±0.02
	DO (mg/L)	0.79±0.02	1.36±0.04	1.80±0.09	2.19±0.16
	Temperature (°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
CHC	pH	7.12±0.09	7.14±0.09	7.19±0.20	7.22±0.11
	DO.(mg/L)	0.80±0.02	1.38±0.04	1.90±0.07	2.39±0.09
	Temperature (°C)	25.20±0.40	26.03±0.31	26.73±1.56	26.80±0.62
	Microbial Count (CFU/mL)	1.02±0.02E+21			1.42±0.04E+21

Table 6: Results for microbial characterization

Bacteria	Colour	Shape	Gram (+)	Gram (-)
Staphylococcus spp.	Purple	Spherical	+	
Streptococcus spp.	Purple	Spherical	+	
Pseudomonas spp.	Pink	Rod-shaped		-
Bacillus spp.	Purple	Rod-shaped	+	
Acinetobacter spp.	Pink	Round-shaped		-

squared = 0.934. Power to detect the effect was 1.000. Significant univariate main effects for media filtration occurred for TDS, F (8, 9) = 76.577, p = 0.000, partial eta square = 0.986, power = 1.000; MR, F (8, 9) =112.781, p = 0.000, partial eta square = 0.990, power = 1.000; TR, F (8, 9) =127.287, p = 0.000, partial eta square = 0.991, power = 1.000 and TPH, F (8, 9) = 13.714, p = 0.000, partial eta square = 0.924, power = 1.000. Pair-wise comparisons among estimated TPH marginal means did not indicate any significant difference in performance across CF, SCF and ACF filters ($p > 0.05$) for the various runs. Pair-wise comparisons among microbial reduction means, TDS reduction means and turbidity reduction means in each instance differed significantly across CF, SCF and ACF ($p < 0.05$).

Monitored parameters

Hydrocarbon biodegradation occurred over a near neutral pH range of 7.04±0.07 to 7.33±0.08, D.O range of 0.79±0.02 to 2.39±0.09 mg/L and temperature range of 25.20±0.40 to 28.40±1.02 °C (Table 5) an observation consistent with that made by Adams *et al*. (2017) and Antwi-Akomeah *et al*. (2018a, 2018b). Maximum TPH degradation occurred at an optimum pH of 7.22±0.11, optimum D.O concentration of 2.39±0.09 mg/L and optimum temperature of 26.80±0.62 °C. These factors were essential in ensuring the survival of hydrocarbon-eating microbes. A correspondence between D.O levels and TPH removal was observed. This is suggestive of the active utilization of supplied oxygen by aerobic hydrocarbon-eating microbes and also indirectly indicative of the rate of emulsification/ breakdown of applied oil by hydrocarbon-eating microbes. Aerobic hydrocarbon-eating microbes per the temperature range recorded comprised psychrotrophic and mesophilic microbes. These psychrotrophic and mesophilic microbes were essentially of bacteria genera per the recorded pH range (Atlas, 1981; Leahy and Colwell, 1990). The slightly alkaline pH conditions recorded in the present study may be indicative of the production of alkaline intermediates (Antwi-Akomeah *et al*., 2018a, 2018b). Table 5 represents results for monitored parameters.

Hydrocarbon-eating bacteria

Several studies advocate for the application of mixed microbial consortia towards achieving substantial or complete mineralization of a vast spectrum of hydrocarbon contaminants. Mixed microbial consortia have been established to offer broad catalytic or enzymatic diversity for the degradation of various hydrocarbon compounds unlike their individual/pure cultures that can degrade

a narrow range of hydrocarbon compounds. This is further buttressed by the fact that strains of different genera of microbes have been found at sites contaminated with petroleum, a possible indication that each strain or genera has a specific role in the hydrocarbon mineralization process (Atlas, 1981; Joutey *et al*., 2013; Leahy and Colwell, 1990). Gram staining reaction showed the involvement of Gram positive cocci, Gram-positive bacilli, Gram-negative cocci and Gram-negative bacilli in the biodegradation of hydrocarbons. Mixed microbial consortium applied in the present study were found to include but not limited to *Staphylococcus spp.*, *Streptococcus* spp., *Pseudomonas* spp., *Bacillus* spp. and *Acinetobacter* spp. (Table 6).

CONCLUSION

The study essentially established the successful application of selected plant materials namely bamboo and coconut husk chips (essentially wastes) as support substrates for microbial attachment towards hydrocarbon degradation in a water medium. Biodegradation largely depended on significant interaction between applied support media and microbial consortia at play as established statistically. Media filtration following biodegradation proved efficient in further reducing TPH levels, microbial load, TDS and turbidity considerably. The study proved efficient in treating crude oil contaminated water considerably in a short timescale of seven days. The present technology compared to existing conventional treatment techniques achieved TPH removals via three main mechanisms- namely aerobic degradation, anaerobic degradation and media filtration per the setup design. Virtually little is known about the synergized impact of these mechanisms. The current technology can potentially be applied in restoring the health of water resources contaminated with a wide range of organic contaminants. The current technology thus presents a viable treatment option in terms of design, ease of operation, cost and efficiency.

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CONFLICTS OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript.

ABBREVIATIONS

ACF	Activated charcoal embedded ceramic filter
BC	Bamboo chips
BR	Blank run
BSM	Biofilm support media
CF	Ceramic filter
CFU	Colony forming unit
CHC	Coconut husk chips
cm	Centimeter
DO	Dissolved oxygen
df	Degrees of freedom
Eq.	Equation
$FeSO_4.7H_2O$	Iron (II) sulphate heptahydrate
FID	Flame ionization detector
Fig.	Figure
g	Grams
GC	Gas chromatograph
h.	Hour
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
km	Kilometer
L	Liter
m^3	Cubic meter
MANOVA	Multivariate analysis of variance
min.	Minute
mg	Milligram
mg/L	Milligram per liter
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MI	Microbial growth increment
mL	Milliliter

MR	Microbial load reduction
$Na_2HPO_4.2H_2O$	Sodium phosphate dibasic dihydrate
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
NH_4Cl	Ammonium chloride
NTU	Nephelometric turbidity units
ppm	Parts per million
SCF	Sand embedded ceramic filter
Sig.	Significant
SE	Standard Error
TDS	Total dissolved solids
TPH	Total petroleum hydrocarbon
TR	Turbidity reduction
^oC	Degrees Celsius
%	Percentage

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