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Enhanced Bioremediation of Petroleum Contaminated Wetland Sediment Using a Microbial Fuel Cell System

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Abstract:- This research study was designed to carry out biolelectrochemical remediation of petroleum contaminated wetland sediment obtained from Ekerekana-Ama Creek, Okrika, in Rivers State. Sediment Microbial Fuel Cells (SMFCs) consisting of an anode embedded in the anaerobic sediment containing petroleum hydrocarbon contaminants and a cathode suspended in the overlying aerobic water were used. Seven 300 ml sterile transparent bottles labelled T_1 to T_7 subjected to different treatments were used for bioelectrochemical remediation study. Gas Chromatography Mass Spectrophotometric analysis was done to check the level of reduction in petroleum hydrocarbon contaminants at the beginning (0 day) and end (80 days) of bioremediation. Percentage reduction in total petroleum hydrocarbon contaminant (272.63 ppm) in the sediment were 22.42 %, 65.80 %, 6.12 %, 21.70 %, 52.4 %, 27.70 %, 19.93 % for T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. Distinct bacteria isolated were characterized biochemically and molecularly. The identity of the bacteria nucleotide sequences (genus to species) as shown by Basic Local Alignment Search Tool (BLAST) identified *Clostridium sporogenes* (MF623797), *Desulfobulbus propionicus* (MF623798), *Ewingella Americana* (MF623799), *Bacillus amyloliquefaciens* (MF623800), *Helicobacter sp.* (MF623801), *Alcaligenes faecalis* (MF623802), *Clostridium botulinum*, *Bacillus subtilis* (MF623803), *Klebsiella oxytoca* (MF623804), and *Burkholderia cepacia* (MF623805) associated with T_1 to T_7. These isolated microorganisms interplayed metabolically to bring about remediation of petroleum hydrocarbon using a microbial fuel system.

I. INTRODUCTION

Petroleum hydrocarbon contamination in soil and ground water is a wide spread environmental problem especially in the areas with high industrial activities (Du *et al.*, 2011).

Indeed, petroleum industry contributes immensely to aquatic environmental degradation and pollution occurring in Niger Delta area of Nigeria. In this region, oil from the petroleum industry enters the aquatic environment through several sources including fall outs from gas flaring, disposal of used lubrication oils, washings from oil tanks, leakages from marine vessels and out board engines, sabotage, erosion and run off from crude oil polluted lands, seepage, refinery effluents, rupture of ill maintained flow lines/installations, maintenance and engineering errors. The un-burnt hydrocarbons resulting from incomplete combustion of fuel are freely discharged into air from which they can be washed by the rain and back to land and also into the sea adding to continual pollution of the aquatic medium of the area.

Many petroleum pollutants are difficult for microorganisms to degrade under anaerobic conditions as a result of limited electron acceptors in the sediment. The cost of aeration in order to supply electron acceptors for bioremediation is not sustainable. Alternative electron acceptors such as Iron III oxides, sulfates, nitrates into contaminated sediments can stimulate anaerobic oxidation of hydrocarbons but the use of these electron acc eptors can be reduced under anoxic conditions, or may cause secondary contaminations (Huang *et al*., 2011).

Therefore, a less-energy-input technology that will bring about anaerobic oxidation of reduced petroleum hydrocarbons in the water logged soils or sediments is best option. Sediment Microbial Fuel cell (SMFC) is a bioeletrochemical system where anodic electrode pulls out liberated electron during anaerobic oxidation of organic pollutants in sediment into air-cathode electrode where reduction reaction occurs leading to bioremediation.

In sediment microbial fuel cells (SMFCs) otherwise known as benthic microbial fuel cell (BMFC); microorganisms are used as biocatalysts to oxidize biodegradable organic substrates such as petroleum hydrocarbon in the sediment and transfer electrons to the anode embedded in or rested on top of the sediment, and then the electrons are transferred to the cathode suspended in the overlying seawater, where electron and proton chemically combine with dissolved oxygen in a reductive reaction leading to production of water (Donovan *et al*., 2011).

In comparing sediment microbial fuel SMFC with other types of microbial fuel cells, Sediment Microbial Fuel Cell system is one of the membranes-less bioelectro-chemical systems designed to eliminate pH imbalance.

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A Proton Exchange Membrane (PEM) has been found to have slow proton transfer capacity and could result in a rapid accumulation of acidity in the anode, which can decrease the activity of exoelectrogens (Harnisch *et al*., 2008). Thus, omitting membrane from MFC is an effective way to balance pH in the anode and cathode (Liu and Logan, 2004).

Sediment microbial fuel cell can be employed for sediment bioremediation or as power sources for fresh water or marine studies (Donovan *et al.,* 2008).

II. MATERIALS AND METHODS

➢ *Sample collection:*

Petroleum hydrocarbon contaminated Sediment and sea water used for laboratory investigations were collected from Ekerekana-Ama creek in Rivers State of the Niger Delta where oily activities are predominant. Samples were collected randomly with a mini-shipek grab sampler at a depth of 3 cm from subsurface sediment and sea water into sterile bottle from 0-3 cm surface. Random Samples were homogenized for even distribution of contaminants and to ensure representativeness of the sample area and thereafter transported to the laboratory at 4 °C in ice pack.

➢ *Sediment Microbial Fuel Construction*

Sediment microbial fuel cells as shown in Fig. 1 and Fig. 2 were constructed. Seven (7) sterile transparent empty bottles (350 ml, 265 g) were used and labeled T1 to T7 for set up which were subjected to different treatments.

Each bottle was filled with petroleum hydrocarbon contaminated sediment weighing 1000 g from the sample area occupying about 6 cm height of the bottle.

Ten (10) graphite electrodes obtained from batteries were used as anodes and cathodes and copper wires were also used as conductors in the construction of sediment microbial fuel cell according to Hayat *et al*. (2014).

The copper wires and the electrodes were sanitized with 99 % alcohol so as to minimize or eliminate contamination. Each bottle was filled with 150 ml of sea water (salty) from the sample area which served as the aerobic layer of the microbial fuel cell (MFC) while the sediment serves as the anaerobic layer.

The anode and cathode electrodes to which sanitized copper wire had been connected were introduced into the bottles. The anode was buried into the sediment containing the petroleum hydrocarbon while the cathode electrode was made to stay afloat of the sea water in the aerobic layer. The copper wires from the anode and cathode electrode were connected to Digital Multimeter

➢ *Experimental Setup*

The experiment was conducted and observed for 80 days so as to know the effects of contaminated petroleum hydrocarbon sample, mediators, and NPK-15-15-15 on microbial fuel cells performance. Seven (7) sterile transparent empty bottles (350 ml, 265 g) were used labeled T1 to T7 for set up. T1 was sterilized before installation of MFC and 100 µM of mediators (methylene blue and neutral red) were used according to Taskan *et al.* (2014) for T4 and T5 respectively and 5 g of sterile N-P-K 15-15-15 fertilizer as biostimulant was used for 1000 g sediment in the bottle.

The Treatment for the samples could be summarized as follow:

T1 = sterile (sediment + seawater) + MFC
T2 = Sediment + Seawater + MFC
T3 = Sediment + Sea water
T4 = Sediment + Seawater + Mediator (Neutral Red) + MFC
T5 = Sediment + Seawater + Mediator (Methylene Blue) + MFC
T6 = Sediment + Seawater + NPK + MFC
T7 = Sediment + Seawater + NPK

Fig. 1:- Experimental set up (T1 to T7)

Fig. 2:- Functioning Sediment Microbial Fuel Cells in a Bioelectrochemical remediation

➢ *Total Organic Carbon Determination*

Wet oxidation technique was used to measure total organic carbon as previously reported by Nelson and Sommers (1996). A 0.1 molar concentration of the sample slurry was pipetted into a clean Pyrex conical flask. 5ml of potassium dichromate solution and 7.5 ml concentrated sulphuric acid was added. The mixture was heated on an electro thermal heater for 15minutes to reflux. The sample was cooled to room temperature and diluted to 100 ml with distilled water. 25 ml of the sample solution was titrated with standard ferrous ammonium sulphate using ferrion as indicator. A blank containing oxidant and sulphuric acid was titrated as in the sample and the titre values recorded. The percentage total organic will be obtained by substituting obtained readings into the formula below.

$$\%\,TOC = \frac{\text{Titre value of sample} - \text{sample titre}}{\text{Sample weight x } 0.2 \text{ x} 0.3}$$

➢ *Determination of petroleum Hydrocarbon Species in sample*

Gas Chromatography Mass Spectophotometric analysis of soil sample was conducted using gas chromatography (Agilent Hp 68 90 series ii) coupled to a mass selective detector by electronic impact lionization (Agilent HP 5973) using a HP5-MS(30 m x 0.25 mm x 0.25 NM)Agilent). The operating condition of the chromatograph is as follows: Injector (splitless l minute) 320^{o}c, injection volume 1.3 NL (depending on the sample), oven temperature, 50 ^{o}c (l minute), ramp 7^{o}c l minutes, final temperature 320 ^{o}c, carrier gas Helium at 0.7 for 11 minutes. The detector worked at solvent delay mode (3.2 minute) and the mass range measured was 40-400 (M/Z). The detected hydrocarbon species were identified by comparing the mass spectra with data in the willey 7 ® library.

➢ *Microbiological Analyses*

- *Enumeration of Total Culturable Heterotrophic Bacteria (TCHB) (aerobes and anaerobes)*

Plate count agar (PCA) was prepared according to the specification of manufacturer and sterilized by steam under pressure (i.e. autoclaved). Spread plate technique was used on plate count agar (PCA) to culture the bacteria as previously described by Pelzar *et al*, (2004). 0.1 ml aliquots of appropriate dilutions were spread on duplicates of sterile PCA plates the inoculated plates were incubated for period of 18-24 hours in the incubator at 28^{0}C ± 2^{0}C to observe colonial formation. In the same way but with little modification and extra carefulness; isolation of total heterotrophic culturable anaerobic bacteria was done by using Gaspak to create anaerobic condition for the bacteria during incubation.

Colonies formed during the incubation period were counted using colony counter. Colonies on plates within the range of 30-300 were recognized. The colony forming unit per gram (cfu/g) was calculated with the relation:

cfu/g = No of colonies × dilution factor Volume of inoculum used

- *Enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (aerobes and anaerobes)*

Bushnell-Haas Agar (Sigma-Aldrich, USA) was prepared according to the specification of the manufacturer, sterilized and used in vapour-phase technique as previously reported by Hamamura *et al.*

(2006). Hydrocarbons were supplied through the vapour phase to putative hydrocarbon utilizers by placing sterile Whatman No.1 filter papers impregnated with 5 ml sterile crude oil on the lids of the inverted plates and incubated for 7 days at 30 °C.

In the same way, anaerobic bacteria that are capable of utilizing petroleum hydrocarbon were isolated with sterile BHA with inclusion of Gaspak during incubation so as to create anaerobic condition. Colonies of the organisms were counted as usual.

Moreover, Post gate agar was formulated as previously reported by Wargin *et al.* (2007) with exclusion of sodium lactate and used in vapour phase as in BHA to screen for sulphur reducing bacteria that are bioeletrochemically active and possessing the potentiality of petroleum hydrocarbon utilization.

III. RESULTS AND DISCUSSION

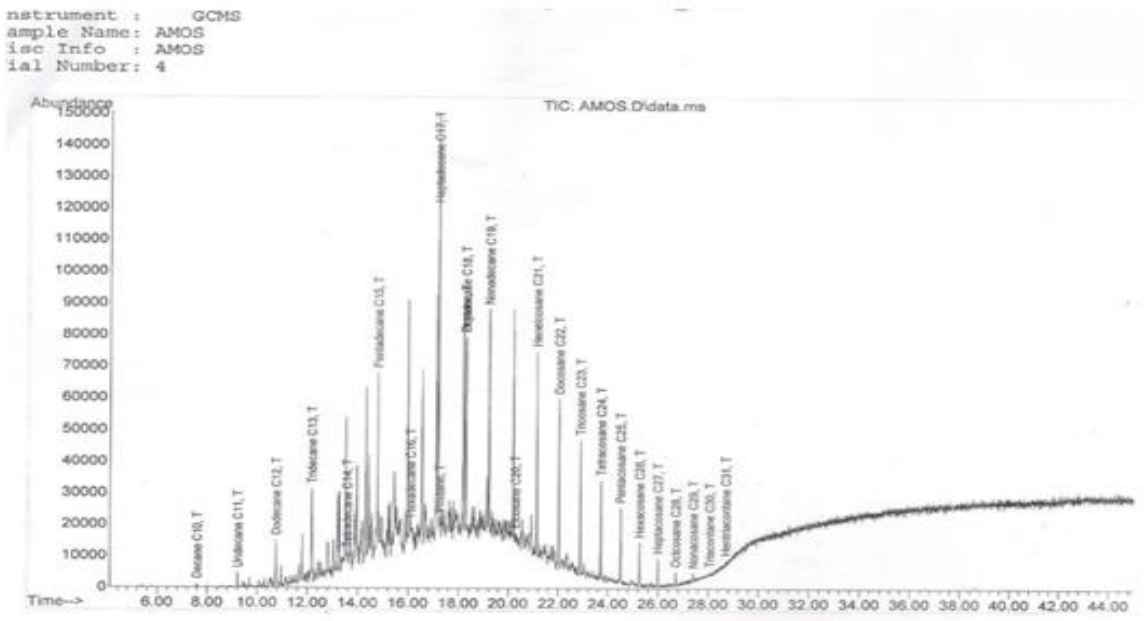


Fig. 3: Chromatogram showing hydrocarbon fractions in the sediment sample under investigation

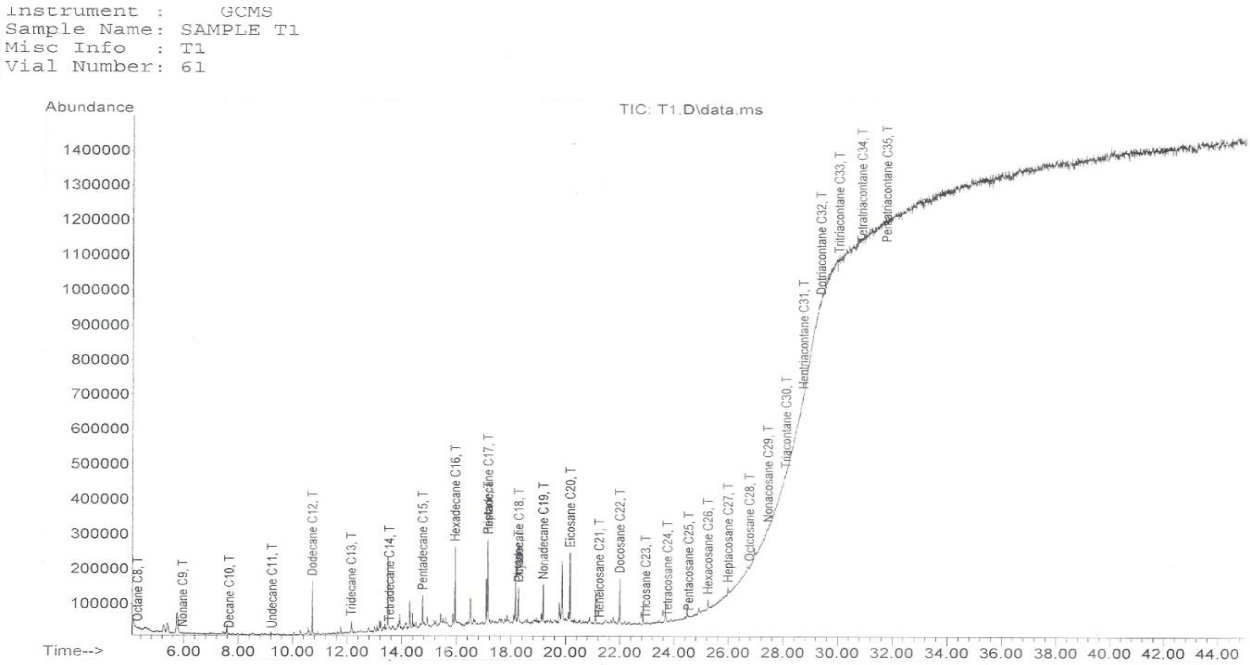


Fig. 4: Residual Petroleum Hydrocarbon Fractions in T_1 at the end of Bioremediation Study

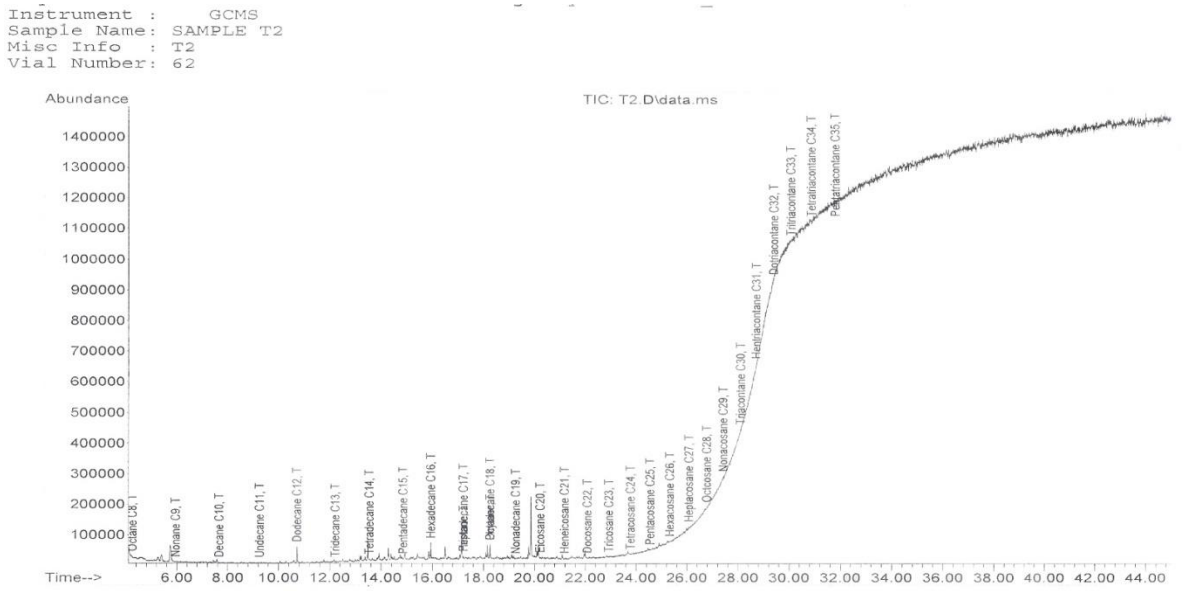


Fig. 5: Residual Petroleum Hydrocarbon Fractions in T_2 at the end of Bioremediation Study

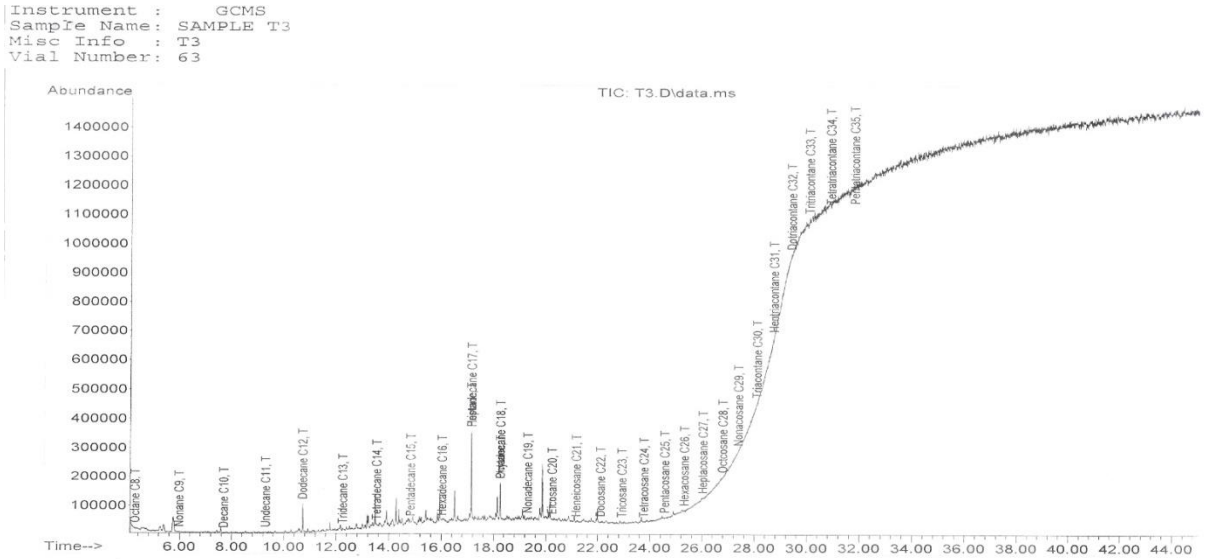


Fig. 6: Residual Petroleum Hydrocarbon Fractions in T_3 at the end of Bioremediation Study

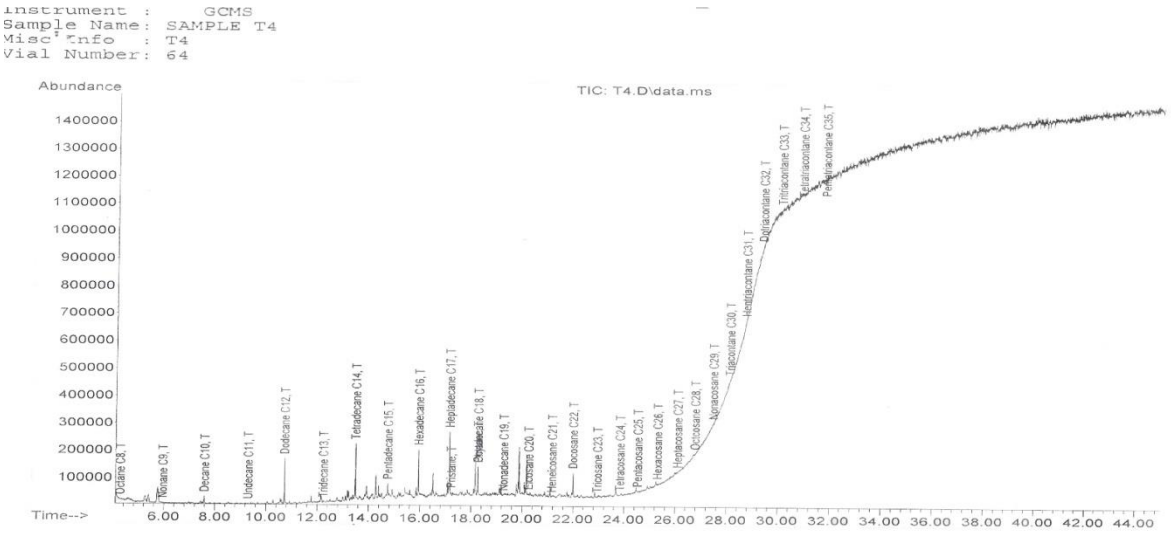
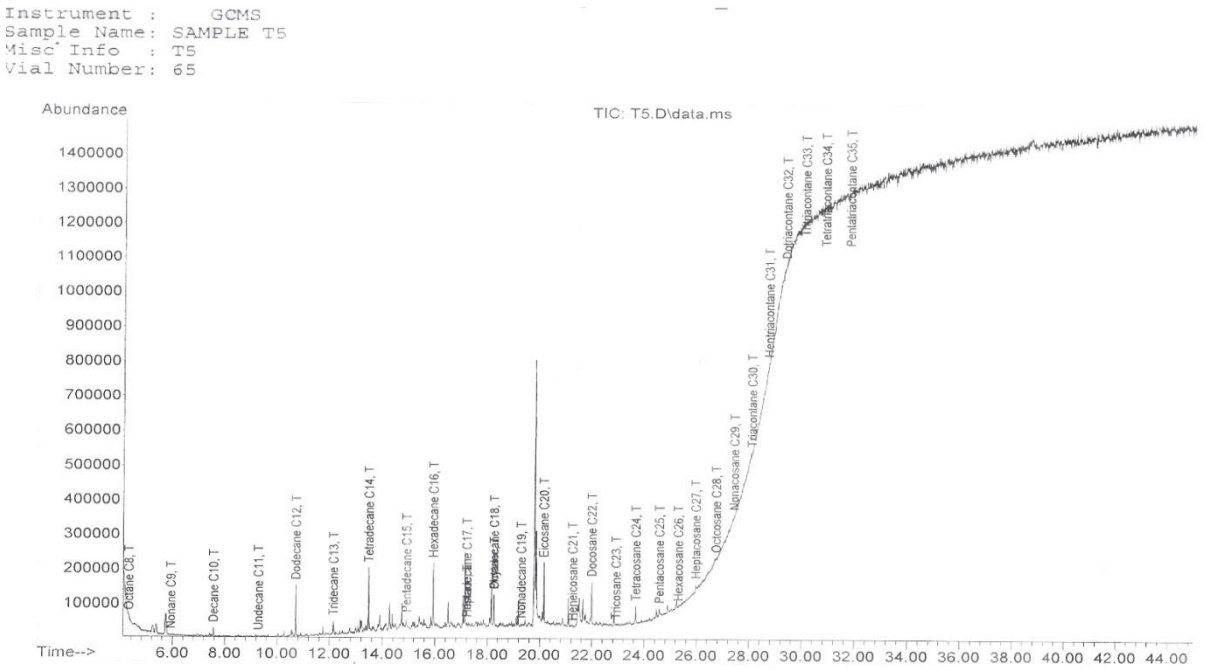


Fig:7 Residual Petroleum Hydrocarbon Fractions in T_4 at the end of Bioremediation Study



Fg.8: Residual Petroleum Hydrocarbon Fractions in T_5 at the end of Bioremediation Study

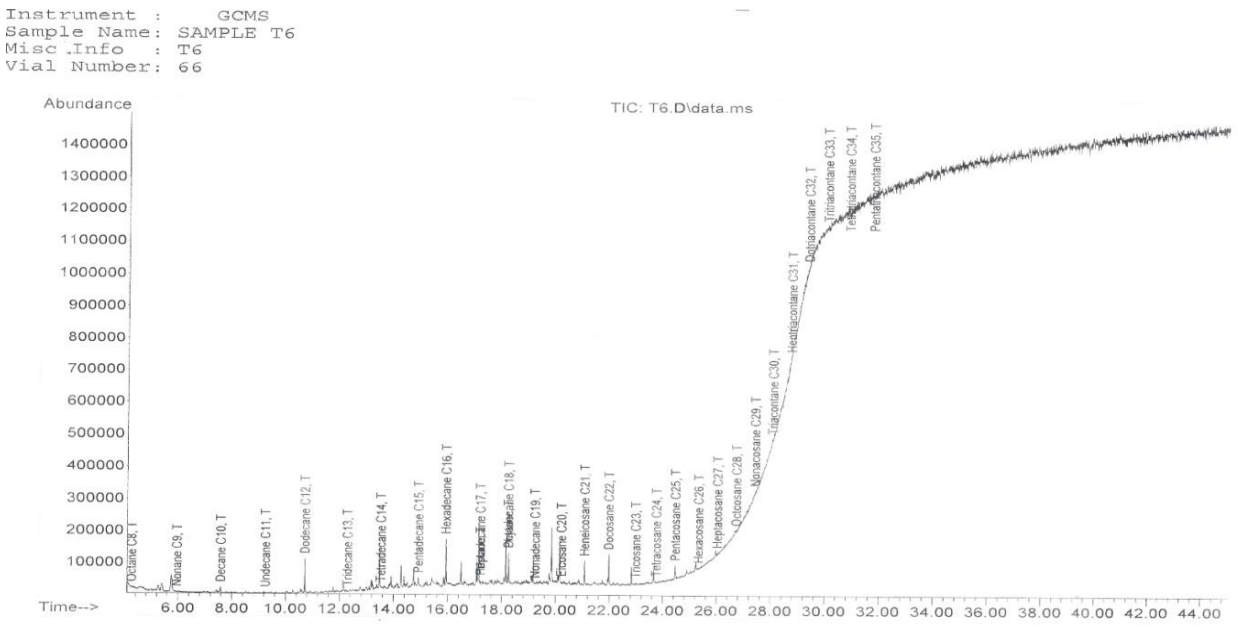


Fig.9: Residual Petroleum Hydrocarbon Fractions in T_6 at the end of Bioremediation Study

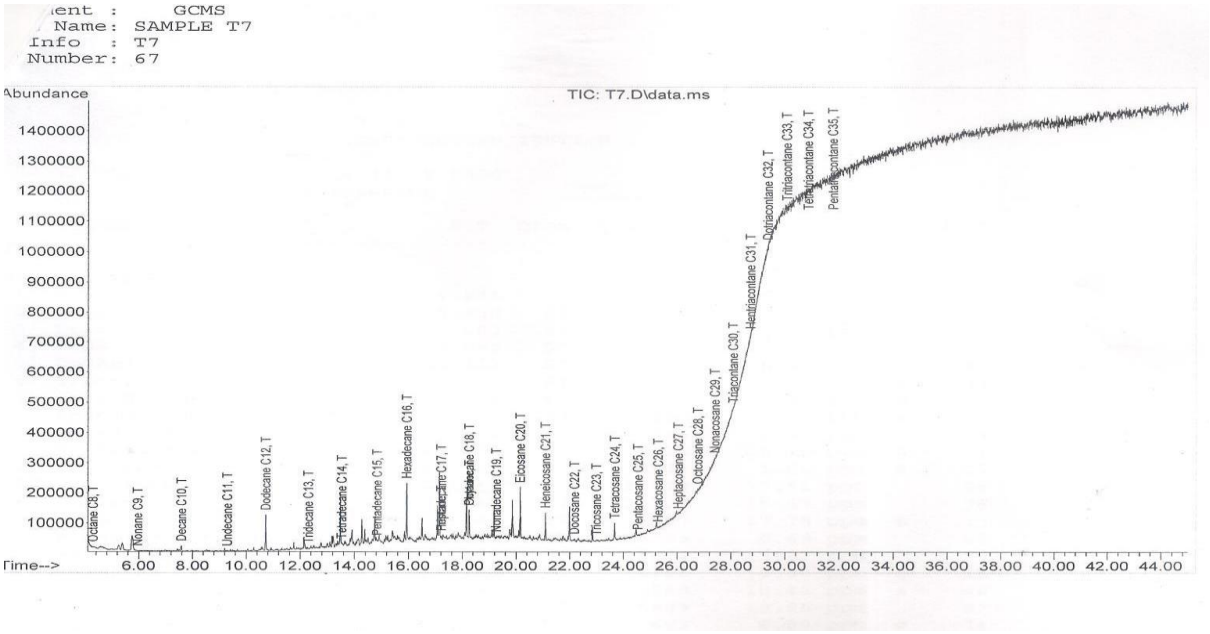


Fig.10: Residual Petroleum Hydrocarbon Fractions in T_7 at the end of Bioremediation Study

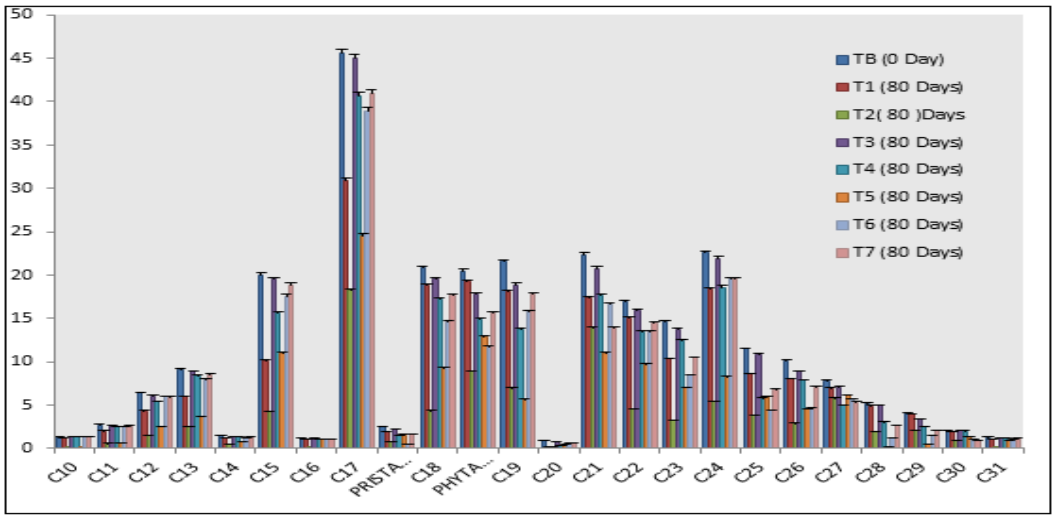


Fig. 11: Changes in Petroleum Hydrocarbon Fractions with Time in sediment subjected to various Treatments during Bioremediation Study

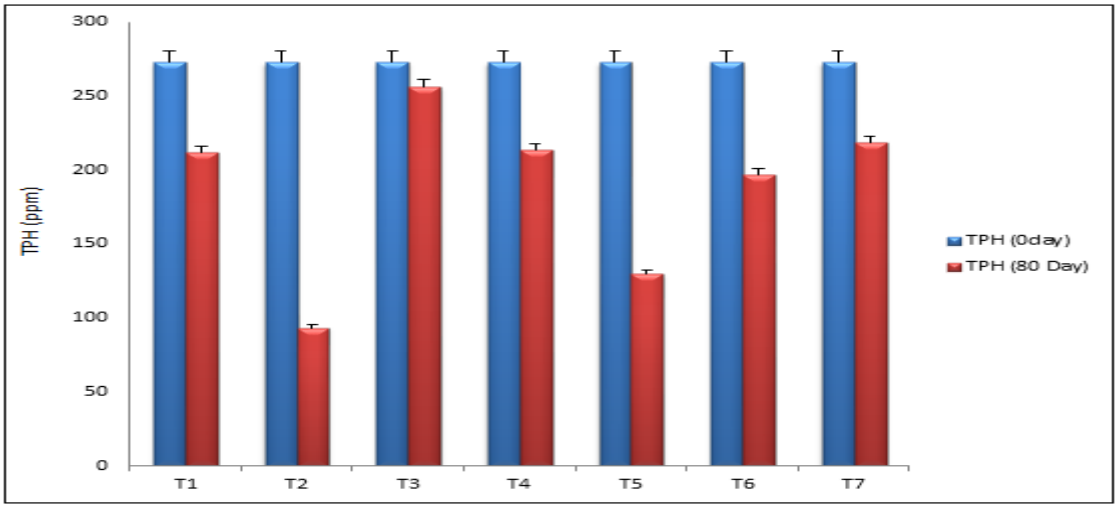


Fig. 12: Changes in TPH (ppm) with Time in Sediment Samples Subjected to Various Treatments during Bioremediation Stud

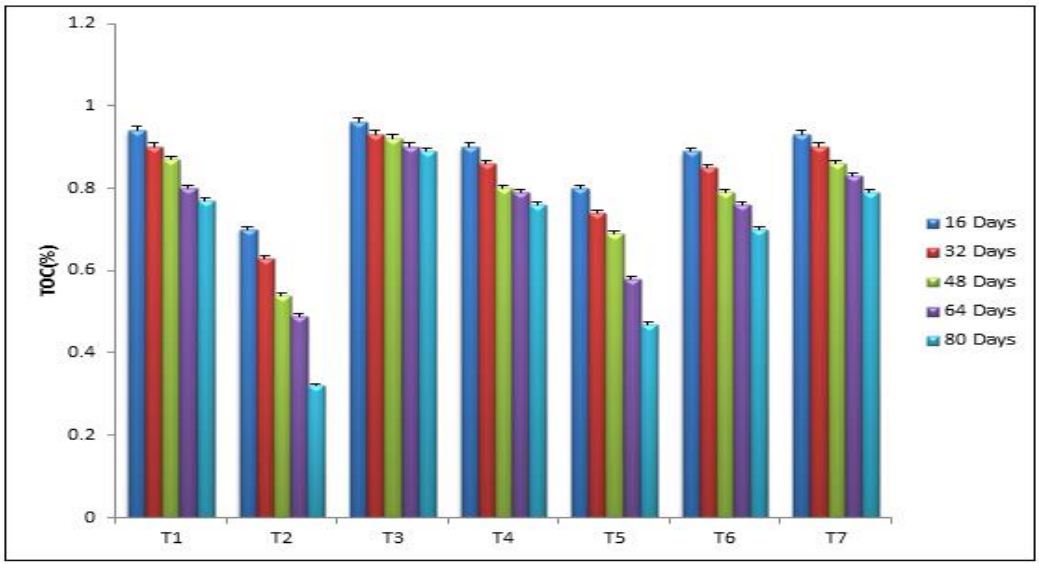


Fig. 13: Changes in TOC (%) With Time in Petroleum Hydrocarbon Polluted Sediment Samples Subjected to Various Treatments during Bioremediation Study

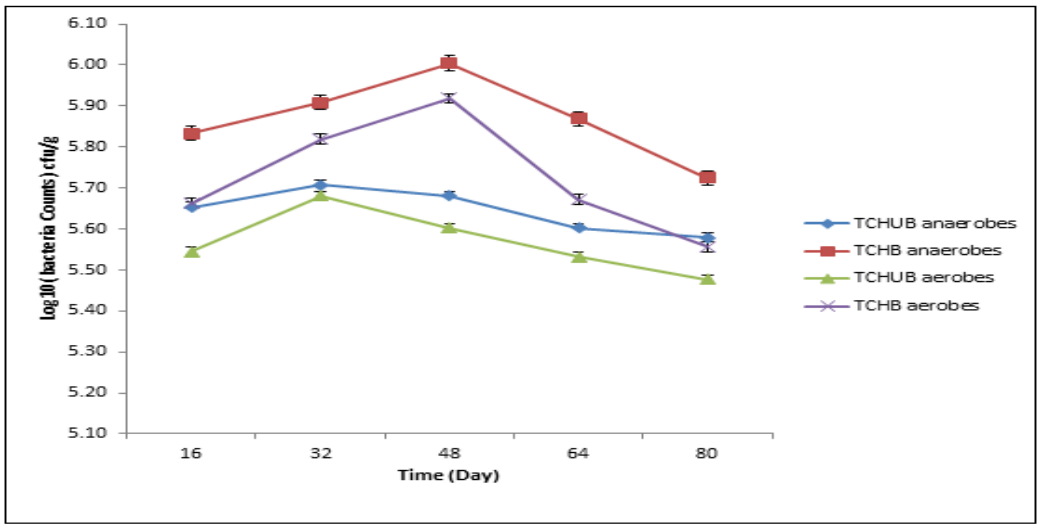


Fig 14: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_1) coupled to MFC during Bioremediation

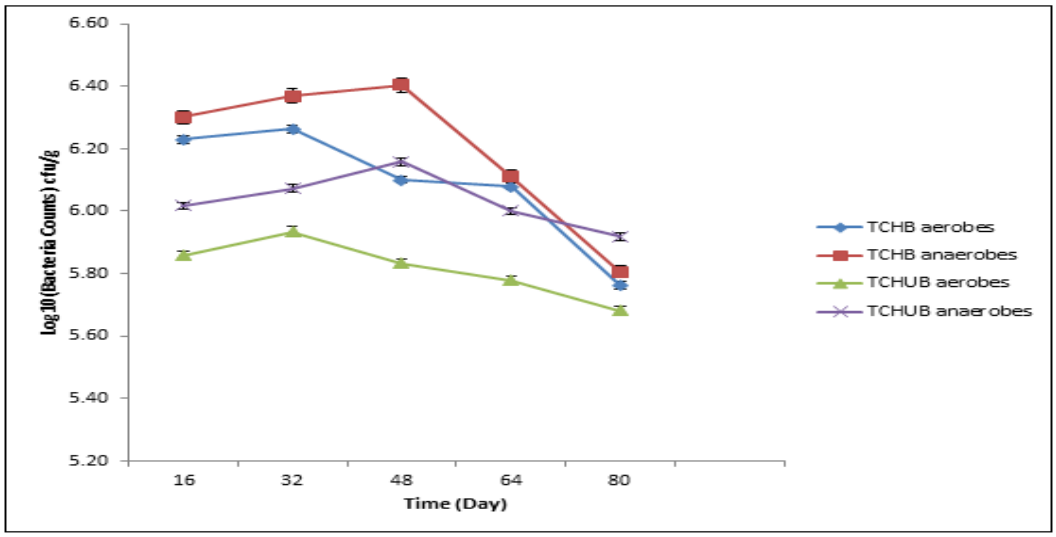


Fig.15: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_2) coupled to MFC during Bioremediation Study

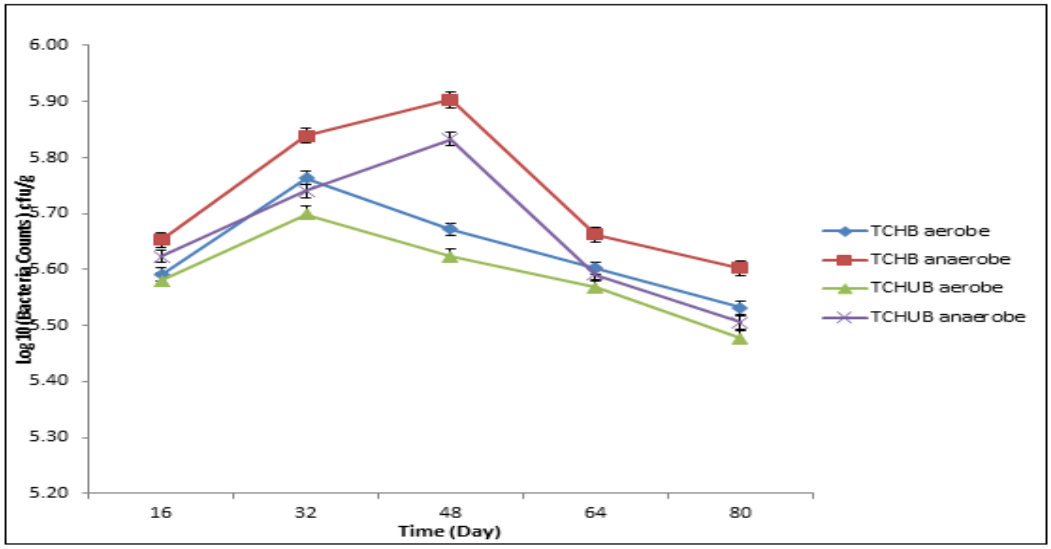


Fig. 16: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_3) without MCF during Bioremediation Study

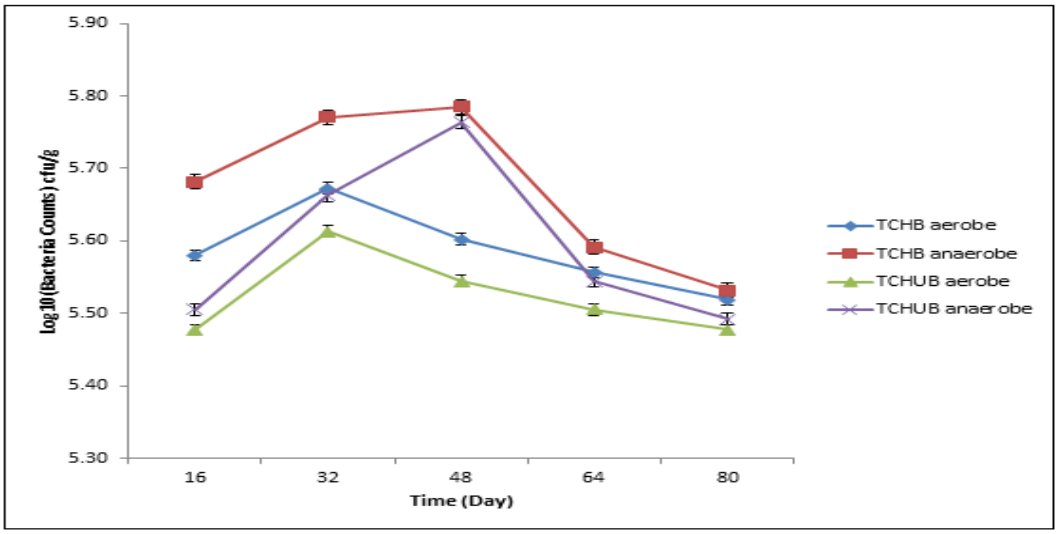


Fig. 17: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_4) coupled to MFC during Bioremediation Study

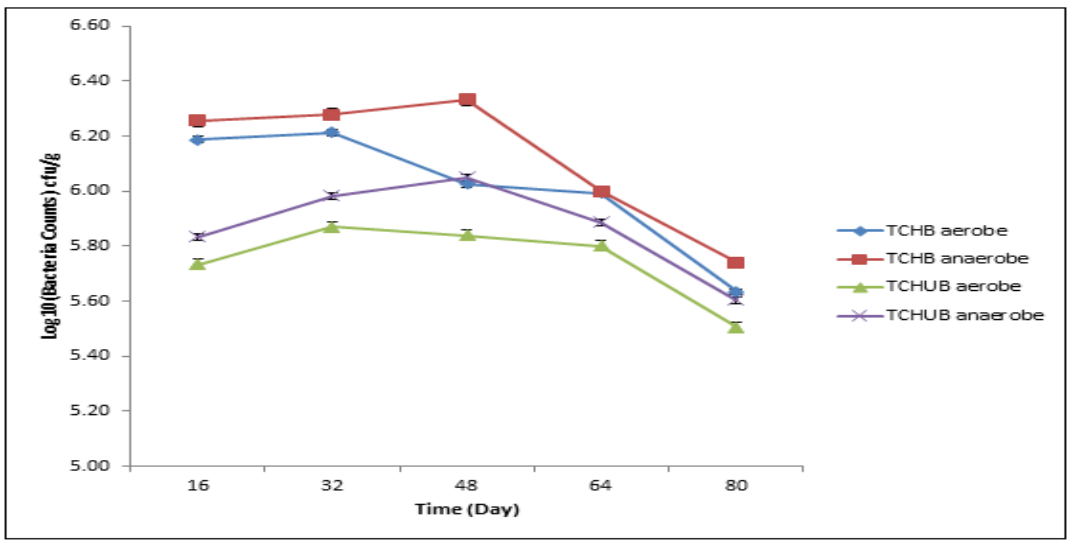


Fig. 18: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_5) coupled to MFC during Bioremediation Study

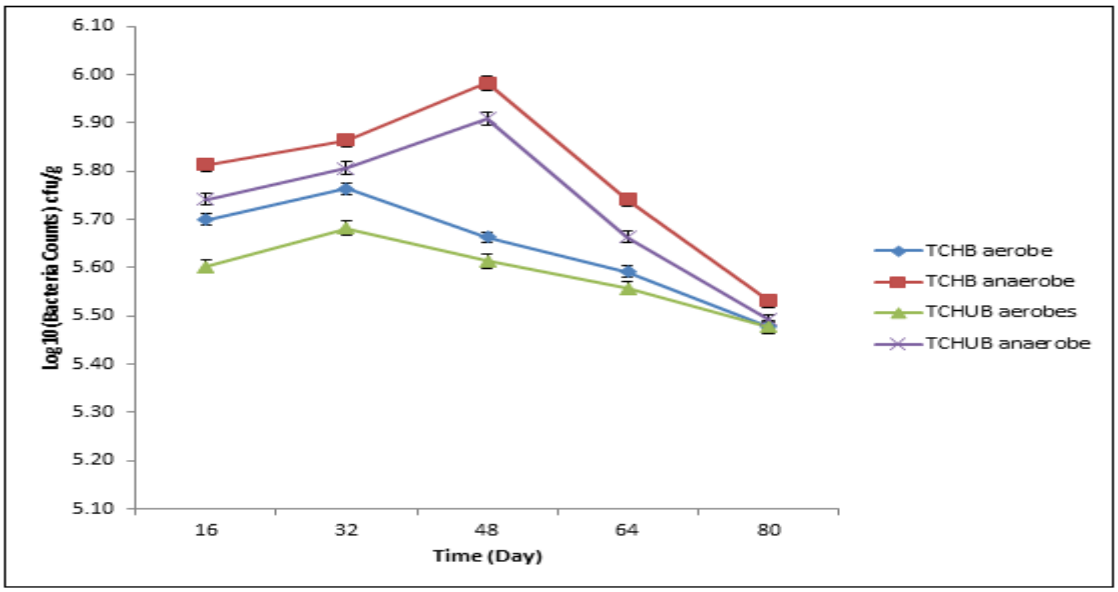


Fig. 19: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_6) coupled to MFC during Bioremediation Study

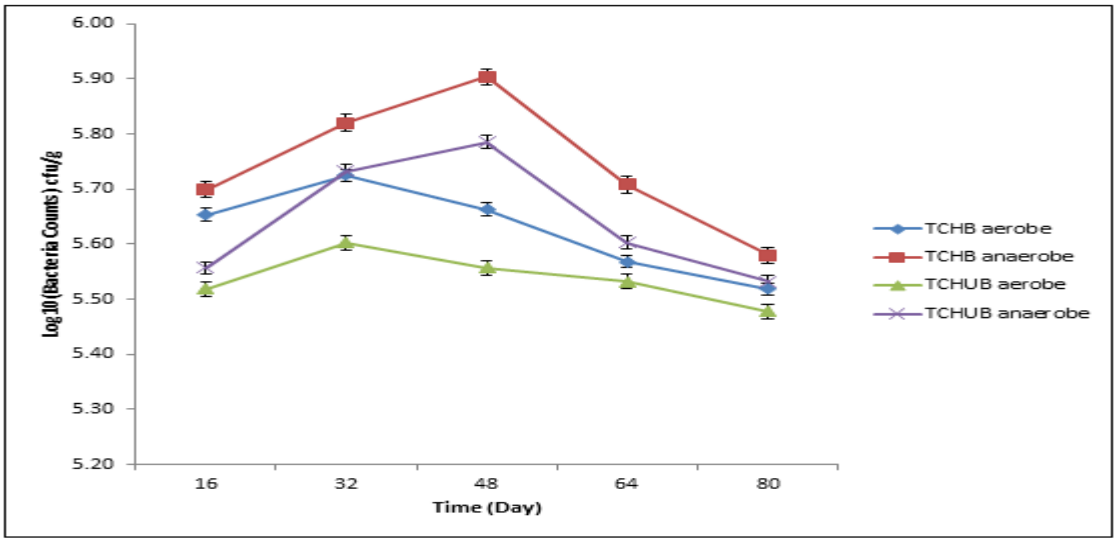


Fig. 20: Changes in Bacterial Population with Time in Petroleum Hydrocarbon Contaminated Sediment (T_7) without MFC during Bioremediation Study

Isolate ID	Gram Reaction	Catalase Test	Oxidase Test	Glucose Metabolism	O-F Test	Spore	Indole	Motility	Tentative identity
A1	+	-	-	+	F	+	-	+	*Bacillus sp.*
A2	-	+	+	-	F	-	-	-	*Enterobacter*
A3	-	+	-	+	F-O	-	-	-	*Bacillus sp.*
A4	-	+	+	+	O	+	-	+	*Enterobacter Sp.*
A5	+	+	+	-	F-O	-	-	+	*Bacillus sp.*
A6	+	+	+	_	O	-	-	+	*Bacillus sp.*
A7	+	-	-	+	F	+	-	+	*Bacillus sp.*
A8	+	+	-	+	O	+	-	+	*Bacillus sp.*
A9	-	+	+	+	F-O	-	+	_	*Serratia sp.*
A10	-	+	+	-	O	-	-	+	*Enterobacter sp.*

Table 1:- Biochemical Characterization of Distinct Hydrocarbon Utilizing Bacterial Isolates obtained from Sediment Samples during Bioremediation Study

+ = positive, - = negative, F= Fermentation, O= Oxidation

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Isolate ID	Blastn Identity	% Identity Similarity	Accession Number
A_1	*Clostridium supergenes* strain DSM 795, complete genome	99	MF623797
A_2	*Desulfobulbus propionicus* strain DSM 2032 16S ribosomal RNA gene, partial sequence	100	MF623798
A_3	*Ewingella americana* strain R12 16S ribosomal RNA gene, partial sequence	89	MF623799
A_4	*Bacillus amyloliquefaciens* strain MBE1283, complete genome	90	MF623800
A_5	*Helicobacter sp.* MIT 01-6242, complete genome	88	MF623801
A_6	*Alcaligenes faecalis* partial 16S rRNA gene, isolate KWW 84	85	MF623802
A_7	*Clostridium botulinum A str.* ATCC 19397, complete genome	99	nill
A_8	*Bacillus subtilis* strain J-5, complete genome	90	MF623803
A_9	*Klebsiella oxytoca* strain CAV1335, complete genome	93	MF623804
A_{10}	*Burkholderia cepacia* strain ATCC 49709 16S ribosomal RNA gene, partial sequence	97	MF623805

Table 2:- Molecular Characterization of Distinct Hydrocarbon Utilizing Bacteria Isolates obtained from Sediment Samples during Bioremediation Study

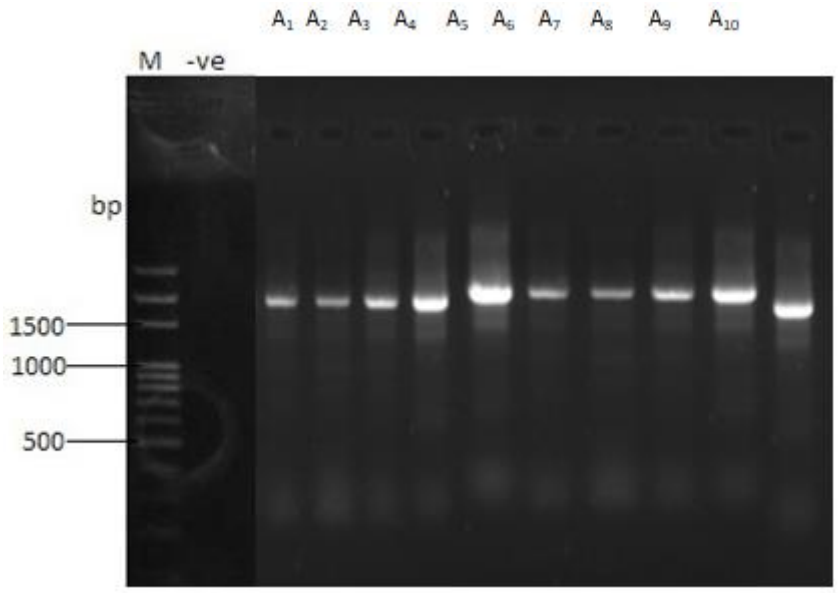

Fig 21:- Gel electrophoresis Photograph of PCR products as revealed by UV Transilluminator

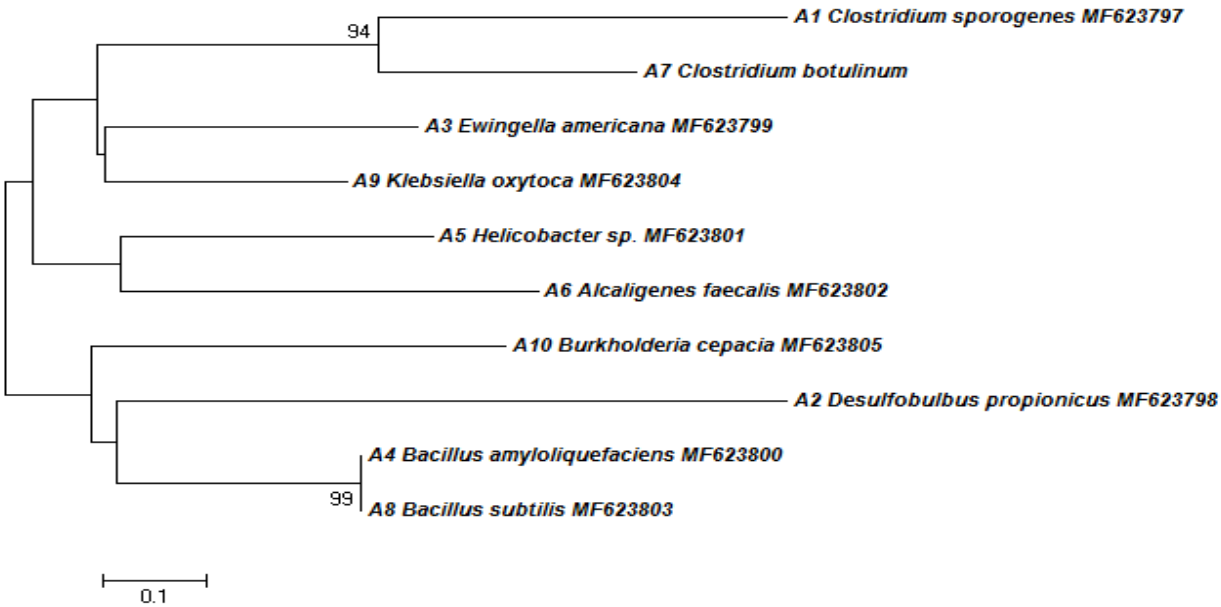

Fig. 22:- Phylogenetic Tree of isolated Bacteria from Sediment Samples (T_1-T_7)

IV. DISCUSSION

In Fig. 11.0, the major branched n-alkane hydrocarbon components in the sediment: pristane (2,6,10,10-tetramethyl pentadecane) -2.48 ppm and phytane (2,6,10,14-tetramethyl pentadecane) - 20.46 ppm as revealed by GCMS were reduced by 66.14 % and 56.50 % in T_2 compared to the control sample (T_3) which has 20.18 % and 6.16 % for pristine and phytane at the end of bioremediation study. Similarly, T_5 containing methylene blue as electron transfer mediator shows 37.10 % and 37.0 % percentage reduction for pristane and phytane. Also, T_4 shows 39.52 % and 27.20 % percentage reduction in pristane and phytane. This implies that The System in T_2 containing MFC even without any mediator enhances utilization of branched n-alkanes by microbial consortia in the system than that of T_5 and T_4 because T_2 contains microbial population that could freely initiate electron transfer through self-mediated system like nanowire or production of exogenous chemical enzymes that could bring about rapid electron transfer to enhance bioremedmdaition. The earlier report from Atlas (1981) on the ability of some microorganisms to utilize and biodegrade branched alkanes which are known to be recalcitrant in environmental aquatic media is really in agreement with this study that pristane and phytane are utilizable in bioelectrochemical system. On the other way, T_6 containing N-P-K 15-15-15 which shows high level percentage reduction of 79.84 % and 42.60 % for pristane and phytane respectively as observed in the study may be that some distinct microbes in T_6 could utilize pristane and phytane in the presence of MFC and NPK 15-15-15 unlike T_7 which does not have MFC but percentage reduction of pristane and phytane as recorded are 35.48 % and 23.41 % respectively.

The TPH baseline value (272.62 ppm) as revealed in the Fig. 12.0 was reduced by 22.42 % in T_1; 65.80 % in T_2; 6.12 % in T_3 (Control Sample). This shows that the MFC in T_2 without any mediator to aid electron transfer enhances bioremediation than that of T_3 which did not provide favourable environment for electrochemically active bacteria (EAB). The dense population of Microorganisms in T_2 contributed to the high level of degradation than that of T_3. However, the sterilization of sediment and seawater in T_1 drastically reduced the population of microbial consortia that could have brought about bioremediation as in T_2.

More so, T_5 containing methylene blue (100 µM) as redox chemical for electron transfer recorded 52.47 % in TPH reduction unlike T_4 which contains Neutral red (100 µM) has percentage reduction in TPH as 21.66 % which shows that Methlyene blue is of good redox mediator than neutral red as reported by Lohar *et al*. (2014). However, in T_6 containing N-P-K 15-15-15, 27.0 % TPH reduction was observed compared to T_2 (65.80 %) and T3 (6.12 %). Although, N-P-K 15-15-15 is known as efficient biostimulator (fertilizer) in bioremediation technology, one should have expected high level of degradation in this sample than any other samples but the degradation observed could be explained by assertion of Vyas *et al.* (2009) of optimum concentrations of N (1 %), P (0.5 %) and K (0.01 %) for biodegradation of crude oil by marine bacteria and that the addition of excess K (0.1 %) proved to be toxic to saltwater bacteria as opined by Kanaly *et al.*(2002) who reported that overloading of oil contaminated sea water or sediments with nutrients inhibit microbial activity due to its toxic effects. Thus, N-P-K formulation of 15-15-15 with equal concentrations of N, P and K could not support high level of degradation in this aquatic system though the formulation may be efficient in other environmental media.

In Figure 12.0, , a progressive decrease in the values of total organic carbon (TOC) from day 16, 32, 48, 64, 80 observed in T_1 to T_6 showed that bioremediation really occurred and this is in agreement with TPH reduction as shown in Fig. 12.0

The heat sterilization of T_1 is observed to have had lethal effects on the microbial population in the system and this affects bioeelectrochemical remediation, however, the spore-forming microbes in the system survived the heat treatment and then developed into fully functional vegetative cells while the microbial population that are not heat resistant died or lost their metabolic viability and cellular integrity. This is supported by Pelczar *et al.* (2004) that numerous microbes have been identified as having resistant ability to heat sterilization. Bacteria spores are much more resistant than vegetative cells. *Bacillus stearothermophilus*, *Clostridium* sp. could withstand temperature of 121 ^{0}C heat for up to 12mins. Endospores of bacteria contain dipicolonic acid which could be responsible for heat resistance and calcium to heat and oxidizing agents.

More so, Fig. 14, Fig.15, Fig.16, Fig. 17, Fig. 18, Fig.19, and Fig. 20 showed microbial growth curves of the total culturable heterotrophic bacteria (TCHB) and total culturable hydrocarbon utilizing bacteria (TCHUB) with sample T_1, T_2, T_3 T_4, T_5 and T_7 respectively. Deduction can be made from the growth curves that anaerobic bacteria population for both TCHB and TCHUB increased till day 48 before a fall in number of the organisms. Whereas, aerobic bacteria population increased till day 32 before progressive decrease in population but decrease in the number of aerobes (obligate and facultative) on day 32 leads to increase in anaerobes (obligate and facultative) proportionally, this may be due to drastic reduction in oxygen concentration in the surface or subsurface region of the sediments as bioremediation proceeds.

Furthermore, Fig.14 shows maximum population of TCHB (aerobe) as 5.10 x10^5 cfu/g which later became minimum value of 3.8 x10^5 cfu/g at the end of bioremediation and maximum bacteria population of 1.01 x10^6 cfu/g was obtained for anaerobic population that are heterotrophic TCHB (anaerobes) with minimum value of 5.30 x10^5 cfu/g. In the same way, TCHUB (aerobe) has maximum and minimum bacteria population of 4.8 x10^5 cfu/g and 3.60 x10^5cfu/g respectively while TCHUB

(anaerobe) highest population of 8.3 x10^5 cfu/g and lowest population of 3.60 x10^5 cfu/g obtained at the end of bioremediation. But, bacteria population in T_2 as shown in Fig. 15 indicates TCHB (aerobe) as 1.83 x10^6 cfu/g, 5.8 x10^5 cfu/g for maximum and minimum population respectively during bioremediation whereas THCB (anaerobe) population of 2.34 x10^6 cfu/g and 6.4 x10^5 cfu/g obtained for maximum and minimum and THUB (aerobe) population of 8.6 x10^5 cfu/g and 4.8 x10^5 cfu/g obtained for maximum and lowest values. THUB (anaerobe) population of 1.44 x10^6 cfu/g and 8.3 x10^5 cfu/g for highest and lowest microbial population. Comparing the bacteria population in T_1 and T_2, it can be deduced that the bacteria population in T_2 is higher than T_1. The higher population in T_2 contributes to bioremediation in T_2 than T_1 and the like. Subjection of T_1 to preheat treatment contributed to lower bacteria population available for bioeletrochemical processes.

Comparing bacterial population in T_3 where TCHB (aerobe) of 5.8 x10^5cfu/g and 3.4 x10^5cfu/g obtained as highest and lowest bacterial population as shown in Fig. 16, and TCHB (anaerobe) of 8.0 x10^5cfu/g and 4.0 x10^5cfu/g maximum and minimum anaerobic population in the sample. Also, TCHUB (aerobe) of 4.2 x10^5 cfu/g, 3.0 x10^5 cfu/g as highest and lowest population respectively and TCHUB (anaerobe) 6.8 x10^5 cfu/g and 3.2 x10^5 cfu/g as highest and lowest bacterial population. The microbial consortia in T_2 are more than T_3 and T_1 while T_1 is higher than T_3.

Similarly, in Fig.17, bacteria population associating with T_4 TCHB (aerobe) of 4.7 x10^5 cfu/g and 3.3 x10^5 cfu/g obtained as highest and lowest microbial population; TCHB (anaerobe) of 6.1 x10^5 cfu/g and 3.4 x10^5 cfu/g obtained as highest and lowest microbial population ; TCHUB (aerobes) of 4.1 x10^5 cfu/g (max. value) and 3.0 x10^5 cfu/g (min. value) ; TCHUB (anaerobes) 5.8 x10^5 cfu/g as highest and 3.1 x10^5 cfu/g as lowest . One can deduce that T_4 population is lower than T_3 due to adverse effect of neutral red on some microbes in the medium.

More so, Fig. 18 shows bacteria population associating with T_5 sample. TCHB (aerobe) of 1.63 x10^6 cfu/g as highest and 4.30 x10^5 cfu/g lowest population at the end of bioremediation; TCHB (anaerobe) of 2.15 x10^6 cfu/g obtained as highest and 5.5 x10^5 cfu/g lowest population. TCHUB (aerobe) of 7.40 x10^5 cfu/g (highest) and 3.20 x10^5 cfu/g (lowest); and TCHUB (anaerobe) of 1.12 x10^6 cfu/g and 4.0 x10^5 cfu/g obtained. Moreover, the bacteria population in T_5 is higher than T_1 and T_3 due to the fact that methylene blue enhances rapid electron transfer and bacterial growth. The dense population of microbes in T_5 contributes to higher remediation due to interdependent metabolic activities common with microbes such as cometabolism.

Similarly, Fig. 19 shows bacteria population associating with T_6 sample. TCHB (aerobe) of 5.8 x10^5 cfu/g obtained as highest and 3.00 x 10^5 cfu/g as lowest population; TCHB (anaerobe) of 9.60 x10^5 cfu/g obtained as highest and 3.40 x10^5 cfu/g as lowest population; TCHUB (aerobe) of 4.80 x10^5 cfu/g (highest) and 3.00 x10^5 cfu/g (lowest) recorded and TCHUB (anaerobe) of 8.10 x10^6 cfu/g and 3.10 x10^5 cfu/g. Bacteria population in T_6 is higher than T_3 without MFC but not as high as that of T_5 and T_2. The Biostimulants in T_6 did not show any significant bacteria growth than other samples except T_3. Also, according to Fig. 20, bacteria population associating with the sample recorded TCHB (aerobe) of 5.30 x 10^5 cfu/g as highest and 3.30 x 10^5 cfu/g as lowest population. Also, TCHB (anaerobe) of 8.00 x10^5 cfu/g obtained as highest and 3.80 x10^5 cfu/g lowest population. TCHUB (aerobe) of 4.00 x10^5 cfu/g (highest) and 3.00 x10^5 cfu/g (lowest); TCHUB (anaerobe) of 6.10 x10^6 cfu/g and 3.40 x10^5 cfu/g were obtained at the end of bioremediation. Comparing T_6 and T_7, one can deduce that NPK 15-15-15 in both samples did show significant bacteria growth. This is supported by Qudot *et al.* (1998) and Chainean *et al.* (2005) who affirmed that high N-P-K levels have negative effects on biodegradation of hydrocarbons and bacteria population of oil degraders. Table 2.0 showed the hydrocarbon utilizing bacteria that were isolated from T_1 to T_7.

Clostridium sporogenes strain DSM 795 (MF623797) and *Clostridium botulinum* A str. ATCC 19397 were isolated from T_1 and T_5 sample. *Clostridium sp.* is known as anaerobic bacterium capable of forming spores. Clostridium spores are highly resistant to heat and may remain dormant (inactive) for some weeks before growth resumption. *Clostridium* has been reported to utilizing petroleum hydrocarbon. Jiang *et al.* (2016) isolated eleven species of *Clostridium* including *C. sporogenes and C. botulinum* from contaminated soil with organic compounds, this is in agreement with this investigation. Three (3) organisms were isolated from T_2. They are: *Desulfobulbus propionicus* strain DSM (MF623798), *Ewingella americana* strain R12 (MF623799), *Bacillus amyloliquefaciencs* strain MBE 1283 (MF623800)

D. propionicus has the metabolic potentialities to utilize organic substrates in sediments. The isolation of sulfate reducing bacteria in this study showed that the sediment is rich in sulfates as supported by the baseline physicochemical features of the sediment.

Two species of *Bacillus* were isolated from this study. They are *Bacillus amyloliquefaciencs* strain MBE 1283 (MF623800) from T_1 and Bacillus *subtilis* strain J-5 (MF623803) from T_5. Both species of *Bacillus* petroleum hydrocarbon remediation. This deduction is supported by Jude *et al.* (2015) in which *Bacillus amyloliquefaciencs* was isolated from mud/soil substrate contaminated with hydrocarbon contaminants resulting to remediation of petroleum hydrocarbon *B. amyloliquefaciencs is* capable of producing biosurfactant containing both hydrophilic and hydrophobic moieties in their structure which can reduce both surface and intersurface tension, hence, facilitating emulsification process in petroleum hydrocarbon degradation.

Bioelectrochemcial potentials of *B. amyloliquefaciencs and B. subtilis* as shown in this study was also supported by Wang *et al.* (2015) where *Bacillus subtilis* and B. *amyloliquefaciencs* were screened for their ability to grow in liquid medium containing petroleum hydrocarbon compound: Diesel, as a sole carbon. Also, Das et al. (2010) isolated Bacillus subtilis among microbial consortia capable of degrading n-alkanes and alkyl aromatics.

Moreover, Olukunle *et al.* (2015) reported *E. Americana* as capable of hydrocarbon degradation by inoculating soil samples containing crude oil with Pure culture of *Ewingella Americana* which increased population of other bacteria consortia after inoculation due to the fact that petroleum components that might be toxic to other organisms were degraded by *Ewingella Americana*. It has been found that *Ewingella Americana* is capable of producing dehydrogenase, this enzyme aids direct electron transfer to electrode in MFC resulting to high current and low redox potential. This assertion corroborates this study that *Ewingella Americana* has contributed to hydrocarbon reduction in sediment microbial fuel cell.

In another development, *Helicobacter* sp. MIT 01-6242 (MF623801) was isolated from T3 which does not contain MFC. *Helicobacter* has be identified as possessing metabolic potency of degrading polyaromatic and saturated hydrocarbons according to widdel *et al.* (2001). Much study still needs to be done to specifically know the species of hydrocarbon that can be degraded by Helicobacter. But *Helicobacter* has not been well known among microbial corsortia in Bioelectrochmeical system. The isolation of *Helicobacter* sp. from T3 which does not contain MFC is still in agreement with available research study so far.

Also, *Alcaligenes faecalis* isolate KWW 84 (MF623802) was isolated from T4 sample. Ezikpe (2009) has reported on the ability of *Alcaligenes faecalis* to utilize chrysene and diesel oil and is also capable of inducing extracellular protein and carbohydrate with concomitant production of biosurfactant for industrial purpose and in bioremediation. Wang *et al*. (2015) noted *A. faecalis* is capable of using releasing electrons from substrates in electrode during denitrification and the production of hydrogen gas by *A. faecalis* in microbial fuel cell as reported by Rabaey *et al*. 2004 is in agreement with this study that Acaligenes can interplay with other microbes to synergistically remediate petroleum hydrocarbon in sediment and generate electricity but the presence of neutral red in T_4 does not really boost electron transfer to commensurate hydrocarbon degradation unlike T5 containing methylene blue.

In the same vein, *Klebsiella oxytoca* strain CAV1335 (MF623804) was isolated from T_6 sample. Klebsiella has the potency to biodegrade organic compounds including petroleum hydrocarbons. This is supported by Islam *et al.* (2016) where *Klebsiella oxytoca* was found to efficiently utilize palm oil mill effluent (POME) in a microbial fuel system. The biodegradation ability of *Klebsiella oxytoca* as observed in this study is also in agreement with Chamka *et al.* (2011) in which *Klebsiella oxytoca* strain degraded crude oil in a Tunisian off-shore oil field and the GCMS analysis showed that *Klebsiella oxytoca* could utilize C_{13} to C_{30} aliphatic hydrocarbons.

Moreover, *Burkholderia cepacia* strain ATCC 49709 was isolated from T_7 sample. *Bukolderia* contributed to the degradation of petroleum hydrocarbon in this investigation as supported by Oyetibo *et al*. (2013) where *B. cepacia* was found to utilize crude oil and anthracene and the bacteria grew in hydrocarbon media amended with Nickel and Cobalt.

V. CONCLUSION

Conclusively, heavily polluted saltwater sediment can serve as source of electron donors for millions of microbes in anoxic sediment. Employment of MFC technology removes needs for aeration and high cost of remediation is reduced. These microbes in anoxic sediments can transfer electron through direct or indirect system leading bioremediation.

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