academicJournals

Vol. 8(6), pp. 401-406, June 2014
DOI: 10.5897/AJEST2014.1707
Article Number: D6652CF45970
ISSN 1996-0794

http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

Full Length Research Paper

Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria

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Received 26 March, 2014; Accepted 5 June, 2014

This investigation was carried out to characterize microbial communities in selected crude oil polluted sites in Bodo community, Gokana Local Government Area of Rivers State, Nigeria. Total heterotrophic bacterial counts ranged from 0.7 to 1.37x10^7 cfu/g and 0.2 to 5.9x10^6 cfu/ml while counts of hydrocarbon utilizing bacteria ranged from 0.1 to 8.0 x 10^6 and 0.2 to 7.5 x 10^5 cfu/ml for soil, sediment and water, respectively. Physiochemical parameters of all samples were determined. The ranges obtained were temperature 31-33°C, pH 7.5-8.2, conductivity 1134 - 7680 µs/cm, total nitrogen 792.4 - 886.3 mg/kg, nitrate 36.55 - 42.70 mg/kg, total organic carbon 2.06 - 2.18%, total petroleum hydrocarbon 1007 - 1104 mg/kg, vanadium 0.001 - 0.007 mg/kg, iron 3.772 - 4.889 mg/kg, chromium 52.40 - 66.20 mg/kg, nickel 40.02 - 41.62 mg/kg, lead 17.30 - 19.40 mg/kg and zinc 35.10 - 39.50 mg/kg for soil and sediments while water had total nitrogen 868 mg/l, nitrate 40.6 mg/l, total organic carbon 3.1 mg/l, total petroleum hydrocarbon 768 mg/l, nickel 39.2 mg/l, lead 17.3 mg/l and turbidity 250 NTU. Bacteria isolates characterized belonged to these genera *Bacillus, Proteus, Pseudomonas, Flavobacterium, Corynebacterium, Serratia, Micrococcus, Klebsiella, Enterobacter* and *Azotobacter.* The findings reveal that there is a high population of active indigenous hydrocarbon utilizing bacteria which can be monitored and enhanced to bring about bioremediation in the study area.

Key words: Hydrocarbon pollution, soil, water, sediments, hydrocarbon utilizing bacteria, Bodo, Ogoniland.

INTRODUCTION

Petroleum is at present, Nigeria's and indeed the world's most important derived energy source (Moffat and Linden, 2005). However the growth and activities of petroleum and petroleum associated industries in Nigeria and in other parts of the world has lead to increased oil pollution in our environment. Crude oil, because of its characteristics is one of the most significant pollutants in the environment as it is capable of causing serious damages to humans and the ecosystem (Okpokwasili, 1996).

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Oil producing areas of Nigeria especially the Niger-Delta area have experienced the devastating consequences of crude oil spills to both terrestrial and aquatic environments in the past 50 years of crude oil exploration and production (Adati, 2012). One of the major reasons for prolonged negative impact of oil spill on the environment could probably be absence of adequate and qualitative scientific baseline data which is required to provide informed and quick response to emergent environmental challenges (Akinde et al., 2012). The Niger Delta is among the ten most significant wetland and marine ecosystems in the world but unsustainable oil exploration activities has rendered the Niger Delta region one of the five most severely petroleum damaged ecosystems in the world (FME, 2006).

Ogoniland is located in the Niger Delta and oil exploration and production activities have been ongoing in this area since the 1950s. Ogoniland is now characterized by oil fields and installations that have remained dormant for several decades. Past spills, lack of maintenance, oil trapping and damage to oil infrastructures have been a common sight in this region and the environment has been without remediation or partially remediated over the years. UNEP report (2011) concluded that pollution of soil by petroleum hydrocarbons in Ogoniland is extensive in land areas, sediments and swampland. The investigation also showed that the surface water throughout the creeks contains massive hydrocarbons.

Hydrocarbons interact with the environment and micro-organisms determining the fate of the contaminant relative to their chemical nature and microbial degra-dative capabilities respectively. Provided the polluted has requisite values for environmental factors that influence microbial activities and there are no inhibitors of meta-bolism, there is a good chance that there will be a viable and active population of hydrocarbon utilizing micro-organisms in the environment (Chikere et al., 2011, 2012b). Considering the large quantity of oil going into the Niger Delta environment especially farmlands and rivers, the need to cleanup crude oil contaminated sites has become a key environmental issue (Vincent et al., 2011).

Conventional methods such as physical removal are the first response option. It is worthy to note that they do not achieve a complete cleanup of the oil spills. Current mechanical methods typically recovers not more than 10-15% of crude after a major spill and almost always leaves the receiving body in worse conditions (Abu and Dike, 2008).

Due to the abilities of certain microbes to mine-ralize hydrocarbon components into environmentally friendly substances such as carbon dioxide and water, the ability of bacteria in breaking down hydrocarbons has gained growing attention in modern day research (Wackett and Hershberger, 2001; Kadali et al., 2012). Biodegradation by microbes is the key removal process of hydrocarbons which is controlled by hydrocarbon physicochemistry, environmental conditions, bioavailability and the presence of catabolically active microbes (Stroud et al., 2007).

This study was conducted to ascertain the microbial diversity associated with the chronically oil-inundated Bodo community in Ogoniland and to some extent ascertain their natural propensity to utilize petroleum hydrocarbons.

MATERIALS AND METHODS

Sampling

One sample each of crude oil polluted water, soil and sediment were collected under aseptic conditions from Bodo community in Ogoniland, Rivers State in the Niger Delta using appropriate equipments. Soil was collected at 0-15 cm soil auger into sterile polyethylene bags. Sediment was collected with an Eckman grab while water was collected into sterile bottles. Samples were collected at different parts of each site, bulked for homogeneity and thereafter transported to the laboratory at 4°C in ice pack.

Determination of physicochemical parameters of samples

Physicochemical parameters such as pH, moisture content, nitrate, phosphate, total organic carbon (TOC), turbidity, salinity, temperature, conductivity and heavy metals were determined using methods from APHA (2008).

Chromatographic analysis

Residual total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were extracted from the samples and quantified using gas chromatograph-flame ionization detector (GC-FID).

Enumeration of total heterotrophic bacteria

Total heterotrophic bacterial (THB) counts were determined using spread plate method on plate count agar (PCA). From each sample 1 g or 1 ml was homogenized in 9 ml of 0.85% normal saline using Heindolph vortexing machine. Decimal dilutions (10-fold) of the suspensions were plated out on agar medium and incubated at 30°C for 24 h. The colony forming units were afterwards enumerated.

Enumeration of hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated by a method adopted from Hamamura et al. (2006) which involved the dilutions of appropriate sample suspensions and plating out on Bushnell-Haas agar (Sigma-Aldrich, USA). Hydrocarbons were supplied through the vapour phase to putative hydrocarbon utilizers by placing sterile Whatman No.1 filter papers impregnated with 5 ml Okono crude oil on the lids of the inverted plates and incubated for 14 days at 30°C.

Purification and characterization of hydrocarbon utilizing bacteria

Discreet colonies of different HUB were randomly picked using a sterile inoculating wire loop and sub cultured for purification by

Table 1. Physicochemical parameters.

Parameter	Sediment	Soil	Water
pH	7.86	5.40	7.66
Electrical conductivity (µS/cm)	7240	1134	3159
Total nitrogen (mg/kg)	886.25	867.20	672.2
Total phosphorous (mg/kg)	11.2	60.4	4.92
Total organic carbon (%)	2.18	5.3	3.1
Nickel (mg/kg)	41.62	41.5	39.2
Lead (mg/kg)	19.40	17.3	17.3
Zinc (mg/kg)	39.5	36.5	32.4
Salinity (PPT)	NA	NA	19.66
Turbidity (NTU)	NA	NA	250
TPH (mg/kg)	1104	1007	768
PAH (mg/kg)	92.6	53.25	85.56

Total 2. Heterotrophic and hydrocarbon utilizing bacterial counts.

Sample	Mean values of THB	Mean values of HUB
Soil (cfu/g)	$1.0x10^7$	$0.5x10^6$
Water (cfu/ml)	$3.1x10^6$	$3.9x10^5$
Sediment (cfu/g)	$2.7x10^6$	$1.0x10^6$

streaking on nutrient agar plates and incubated at 30°C for 24 h. Individual colonies were predominantly identified using biochemical tests as described in Bergy's Manual for Determinative Bacteriology (Holt et al., 1994).

Degradation screening

Representative HUB isolates were further screened for oil degradation capability under aerobic conditions by inoculating a calibrated loop full of 18 h old culture of each hydrocarbon utilizing bacterium into Bushnell Haas Broth containing 1 ml of Okono medium crude oil. Biodegradation was scored by turbidity and emulsification of oil-in-mineral broth medium after 14 days incubation at 30°C (Kostka et al., 2011).

RESULTS AND DISCUSSION

Soil, sediment and water physicochemical parameters are shown in Table 1. The parameters determined indicated that the samples had been exposed to hydrocarbon contamination with traces of other organic and inorganic contaminants (Chikere, 2010, 2012a).

These pollutants cause damages to humans and the ecosystem if not effectively remediated. The contamination may have resulted in the low pH of 5.40 observed in soil as compared to pristine soil while pH in sediments and water were observed to be 7.86 and 7.66, respectively which is neutral to slightly alkaline. Previous studies have demonstrated that the pH range optimal for biodegradation of hydrocarbons is 6-7 (Eweis et al., 1998; Aparna et al., 2010). Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and in some cases iron. Addition of nutrients is necessary to enhance biodegradation of crude oil pollutants.

The presence of microbial activity was determined by the enumeration of culturable total heterotrophic bacteria and total hydrocarbon utilizing bacteria as presented in Table 2 and soil sample recorded highest cfu count for THB with a mean value of 1.0×10^7 cfu/g and sediment highest in HUB count with a mean value of $1.0 \times 10^{6.}$ A similar observation was reported by Ibiene et al. (2011) and Eze and Okpokwasili (2011).

The high counts recorded in soil sample could be attributed to the myriad of nutrients, high organic matter concentration and other ecological factors that influence the survival of soil microorganisms that play important roles in the decomposition and recycling of nutrients. Continuous input of petroleum-based pollutants usually results in an enriched microbial community capable of surviving toxic contamination. The difference between THB and HUB counts was observed to be minimal/-insignificant which suggests that most of the micro-organisms present in the various sample sites are hydrocarbon degraders that can withstand the concentration of crude oil and also use them as source of carbon.

Totally, 47 pure cultures were able to grow on mineral salt medium (Bushnell Haas Agar) with crude oil as carbon source and were identified using phenotypic and biochemical tests. The population of culturable hydrocarbon degraders from soil, water and sediment samples investigated showed that majority of the bacteria were Gram negative belonging to *Gamma proteobacteria* group, this corroborates with the findings of Kaplan and Kitts (2004) although some Gram positive isolates were also observed. A total of 30 isolates had the ability to utilize crude oil and they belonged to the genera *Bacillus, Proteus, Klebsiella, Pseudomonas, Micrococcus, Serratia, Enterobacter, Flavobacterium, Corynebacterium* and *Azotobacter* as presented in Table 3. The flora represents the normal heterotrophic bacteria present in the various samples.

Majority of these organisms isolated were *Proteus*, *Enterobacter* and *Pseudomonas*. These predominant genera isolated have been shown to contain high numbers of oil degrading species from oil polluted sites, and this gives evidence that these species are probably the active degraders in this environment. These isolates have also been demonstrated by other researchers to be hydrocarbon degraders (Sarma and Sarma, 2010; Ebrahimi et al., 2012). Watanabe (2001) isolated *Micrococcus*, *Pseudomonas* and *Bacillus* from sediments as marine petroleum hydrocarbon degraders. Chikere et al. (2009) reported the prevalence of *Flavobacterium*, *Enterobacter, Norcardia* and *Acinetobacter* in a hydrocarbon

Table 3. Characterization of bacterial isolates.

Isolate code	Gram reaction		Tentative identity	Degradative screening
HUBSED1	+	R	*Corynebacterium* sp.	Y
HUBSED2	+	R	*Corynebacterium* sp.	Y
HUBSED3	-	R	*Serratia* sp.	Y
HUBSED4	-	R	*Pseudomonas* sp.	Y
HUBSED5	-	R	*Proteus sp.*	Y
HUBSED6	-	R	*Flavobacterium* sp.	Y
HUBSED7	-	R	*Proteus* sp.	Y
HUBSO1	-	R	*Proteus* sp.	Y
HUBSO2	-	R	*Enterobacter* sp.	Y
HUBSO3	-	R	*Enterobacter* sp.	Y
HUBSO4	-	R	*Flavobacterium* sp.	Y
HUBSO5	-	R	*Pseudomonas* sp.	Y
HUBSO6	+	R	*Bacillus* sp.	Y
HUBW1	+	C	*Micrococcus* sp.	Y
HUBW2	+	R	*Bacillus* sp.	Y
HUBW3	-	R	*Klebsiella* sp.	Y
HUBW4	-	R	*Enterobacter* sp.	Y
HUBW5	-	R	*Serratia* sp.	Y
HUBW6	-	R	*Proteus* sp.	Y
HUBW7	-	R	*Azotobacter* sp.	Y
HUBW8	+	C	*Micrococcus* sp.	Y

Y = Yes, R = rod, C = cocci.

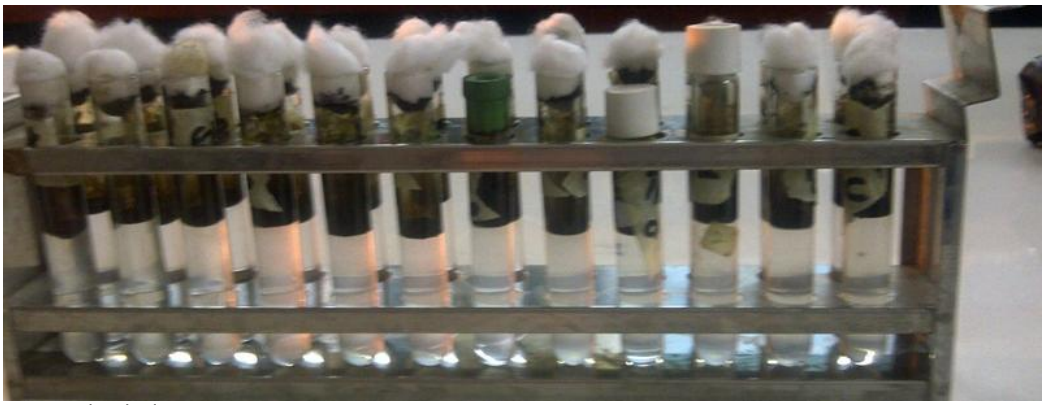
polluted marine sediment undergoing bioremediation. In a related study conducted by Ibiene et al. (2011) at Aluu and Mogho communities in Port Harcourt, Rivers State, Nigeria, *Micrococcus, Bacillus, Corynebacterium, Vibrio, Pseudomonas* and *Flavobacterium,* were isolated from a contaminated soil undergoing bioremediation by natural attenuation. Obire and Nwanbeta (2002) also reported the isolation of *Serratia, Pseudomonas, Proteus, Klebsiella*, *Micrococcus* and *Staphylococcus* species from samples collected from petroleum hydrocarbon contaminated soil in Port Harcourt while Eze and Okpokwasili (2010) isolated *Flavobacterium, Proteus, Bacillus, Klebsiella, Lactobacillus* among other bacteria from Okpoka-Woji river sediment serving as a sink for industrial effluents. Table 3 also indicates that the crude oil degradative ability of the individual bacterial isolates was significant as evidenced by turbidity and emulsification of 1 ml of crude oil in 10 ml of Bushnell-Haas broth after 14 days incubation when compared with the test isolates on day zero incubation as presented in Figure 1. Bacteria that are capable of utilizing hydrocarbons as energy and carbon sources in broth culture have been shown to produce bioemulsifiers or bio-surfactants that assist in the transport of hydrocarbons into the cell via efficient uptake systems (Atlas and Philip, 2005; Olga et al., 2008; Satpute et al., 2010; Cho et al., 2011). Previous studies have demonstrated that the bacterial genera characterized in the present investigation contain known hydrocarbon utilizing species (Chaillan et al., 2004; Brito et al., 2006; Olga et al., 2008; Kadali et al., 2012).

Conclusion

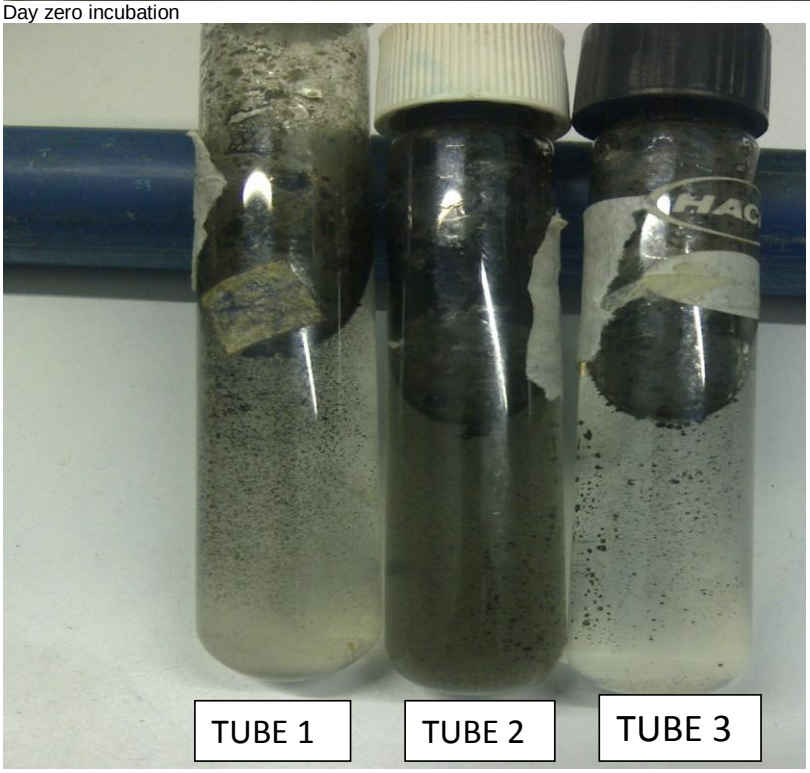
One of the major reasons for prolonged negative impact of oil spill on the environment is probably the absence of adequate and qualitative scientific baseline data. These findings have revealed that there is an appreciable population of indigenous hydrocarbon utilizing bacteria in oil-polluted sites in Bodo which can be monitored and enhanced to increase the bioremediation rate in this chronically oil-impacted area. it is pertinent to study the community dynamics of hydrocarbon degrading bacteria in oil-polluted ecosystems using cultivation-independent 16S rRNA-gene-based and functional-gene-based methods in order to fully undertsand the biochemical reactions that underpin hydrocarbon degradation during bioremediation projects (Chikere, 2013).

Conflict of Interests

The author(s) have not declared any conflict of interests.



Day zero incubation



Day 14 incubation

Figure 1. Biodegradation screening of hydrocarbon utilizing bacteria. Day zero incubation of isolates in test tubes in rack above. Day 14 incubation showing crude oil emulsification by specific isolates. Tube 1: Crude oil degradation by HUBSED2 (*Corynebacterium* sp.); Tube 2: Crude oil degradation by HUBSED4 (*Pseudomonas* sp.); Tube 3: crude oil degradation by HUBSO4 (*Flavobacterium* sp.).

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