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Biodegradation of Total Petroleum Hydrocarbon by a Consortium of Cyanobacteria Isolated from Crude Oil Polluted Brackish Waters of Bodo Creeks in Ogoniland, Rivers State

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ABSTRACT

This study was carried out to determine the capability of isolated cyanobacteria species from crude oil polluted Bodo creek. Isolation of pure cultures of cyanobacteria was done and the isolates obtained were identified molecularly on the basis of 16S rRNA gene sequence analysis. The 16S rRNA sequencing for identification of the isolates generated sequences ranging from $\geq$600 bp and a 250 bp size PCR amplified fragment. The nucleotide sequence of the 16S rRNA gene was compared with published 16S rRNA sequences using BLAST search at the data base of National Center for Biotechnology Information (NCBI). Biodegradation of petroleum hydrocarbon by the isolates was monitored using GC-FID (Agilent 6890 model) for 49 days. The initial quantity of TPH was 29882 and 753.7 mg L^{-1} on day 0 and day 49, respectively. Loss of TPH was statistically significant using one way analysis of variance (ANOVA) $p<0.05$ with time.

Key words: Cyanobacteria, biodegradation, total petroleum hydrocarbon, consortium

INTRODUCTION

Petroleum is Nigeria's and the world's most important derived energy source (Moffat and Linden, 2005). Due to growth and activities of petroleum and petroleum associated industries in Nigeria and world over, there have been increased occurrences of pollution in our environment. The characteristics of crude oil has made it one of the most significant pollutant in the environment as it is capable of causing serious changes to humans and the ecosystem (Okpokwasili and Odokuma, 1990; Okpokwasili, 1996). In Nigeria, oil producing areas especially the Niger Delta have a devastating experience of oil spills on both the terrestrial and aquatic environments in the past 50 years of crude oil exploration and production (Kadafa, 2012). The Niger Delta is among the ten most significant wetland and marine ecosystems in the world which ecosystem has been severely damaged by petroleum pollution due to unsustainable oil exploration activities (FME., 2006). Contamination of surface water with petroleum hydrocarbon is reportedly among the most severe environmental problems facing Nigeria (Okoh *et al.*, 2001; UNEP., 2011). Bodo creek is located in Ogoniland of Gokana LGA which is located in the Niger Delta where oil exploration and production activities commenced since 1950's and is now characterized by oil fields and installations that have remained dormant for several decades. Gokana LGA is over the major oil producing areas in Rivers State and is located within the South East senatorial district of the state, comprising both the riverine and upland communities. Past spills, lack of maintenance, oil trapping and damage to oil infrastructures have been common sight in this region and the environment has been without

remediation or partially remediated over the years (Chikere and Ekwuabu, 2014). Investigation of UNEP (2011) revealed that surface water throughout the creeks contains massive hydrocarbons. Ichor *et al.* (2014) reported heavy contamination of Bodo creeks with crude oil due to oil exploration activities and spillages.

Biodegradation denotes complete microbial mineralization of complex minerals into simple inorganic constituents such as carbon (IV) oxide, water and minerals as well as biomass. In aquatic and terrestrial environments, the biodegradation of crude oil and other petroleum complexes predominantly revolves around the actions of bacterial and fungal populations (Alexander, 1999). Biodegradation of hydrocarbons is often relatively slow under normal conditions due to the complex interaction that involves hydrocarbons, the environment and the composition of the microbial community. Hydrocarbons interact with the environment and microorganisms determining the fate of the contaminant relative to their chemical nature and microbial degradative capabilities, respectively through requisite values for environmental factors that influence microbial activities and absence of inhibitors of metabolism will ensure a visible and active proportion of hydrocarbon utilizing microorganisms in the environment (Chikere *et al.*, 2011, 2012; Chikere and Ekwuabu, 2014). Cyanobacteria are oxygen evolving photosynthetic prokaryotes that are known to grow in extreme environments (Thajuddin and Subramanian, 2005). They are also known as blue green algae and are highly genetically diverse occupying a broad range of habitats across all latitudes (Kulasooriya, 2011). They have been recognized as an excellent source of vitamins, proteins and have a great deal of potential as a source of fine chemicals, biofertilizer and renewable fuel (El-Bestawy *et al.*, 2007). The metabolic activities such as photosynthesis performed by cyanobacterial cells can affect the oxygen and pH conditions of the surrounding water and change the amounts of available organic compounds and nutrients as well as abundances of antibacterial compounds (Oliver and Ganf, 2000; Kirkwood *et al.*, 2006). For example, many planktonic cyanobacterial species can fix atmospheric nitrogen and convert it into forms which can be accessed by other organisms.

Previous reports have shown the ability of cyanobacteria to oxidize oil components. Studies on *Oscillatoria* sp. and *Agmenellum quaduplicatum* demonstrated the ability to oxidize naphthalene to 1-naphthol (Cerniglia *et al.*, 1979, 1980). Other studies showed that *Oscillatoria* sp., strain JCM can oxidize biphenyl to 4-hydroxybiphenyl and *Agmenellum quadruplicatum* metabolizes phenanthrene into trans-9,10-dihydroxy-9,10-dihydroxyphenanthrene and 1-methoxy-phenanthrene (Narro *et al.*, 1992). Several other strains were reported to degrade other complex organic compounds such as surfactants and herbicides (Radwan and Al-Hasan, 2000; Raghukumar *et al.*, 2001; Mansy and El-Bestawy, 2002; Hoffmann, 1996; Hopner *et al.*, 1996; Grotzschel *et al.*, 2002) demonstrated that cyanobacterial mats were rich in oil degrading bacterial and were shown to be capable of degrading petroleum compounds. There is no doubt that cyanobacteria play an important role in microbial mat by establishing oxygen gradients and supplying nutrients for heterotrophic bacteria. However, it is yet not clear whether cyanobacteria or heterotrophic bacteria are responsible for biodegradation of oil compounds though the dominance of cyanobacteria in many polluted sites including the contaminated coasts of the Arabian Gulf gave the impression that cyanobacteria possess the potential to break down oil components (Sanchez *et al.*, 2005).

However, it is by no means clear whether oil degradation is carried by cyanobacteria alone or by heterotrophic bacteria associated to cyanobacteria as coresponsible for hydrocarbon degradation. Al-Hasan *et al.* (1998) demonstrated that non axenic cyanobacterial samples containing *Microleus chthonoplastes* and *Phormidium corium* consumed and oxidized n-alkanes. They found that

cyanobacterial growth steadily declined with progressive anxiety and they identified four genera of heterotrophic bacteria (*Rhodococcus rhodochrous*, *Arthrobacter nicotianae*, *Pseudomonas* sp. and *Bacillus* sp.) able to oxidize n-alkanes, although cyanobacteria directly contributed to hydrocarbon uptake and oxidation. On the other hand, Al-Hasan *et al.* (2001) demonstrated that picocyanobacteria from the Arabian Gulf accumulated hydrocarbons from the water body, but did not utilize these compounds; they assumed that associated bacteria may be carrying out the degradation.

In spite of the many reports showing the ability of cyanobacteria to degrade various organic compounds including constituents of petroleum, a convincing proof is still lacking. It is unlikely that cyanobacteria, capable of fixing carbon (IV) oxide by photosynthesis, need to degrade oil components to obtain organic carbon. Heterotrophy which is the ability to use an external carbon source, though it exists, is very rare among cyanobacteria (Radwan and Al-Hasan, 2000). Few cyanobacteria were reported to utilize a limited number of organic substrates mainly sugars such as glucose, fructose, ribose, sucrose as well as glycerol (Rippka *et al.*, 1979; Moore *et al.*, 1979).

The specific aim of the study is to test the ability of cyanobacteria strains isolated from crude oil polluted brackish waters of Bodo creek to degrade crude oil and to isolate and identify them. The objectives of the study include:

- Isolate and identify key cyanobacteria resident populations in Bodo creek using conventional culturing and molecular methods
- Monitor the biodegradation of petroleum hydrocarbons by the consortium of cyanobacteria
- Determine the rate of petroleum hydrocarbon degradation by the isolated consortium of cyanobacteria

MATERIALS AND METHODS

Media and incubation conditions: The BG-11 medium consisting of (g L^{-1}) $NaNO_3$ (1.5), K_2HPO_4 (0.004), $MgSO_4.7H_2O$ (0.0075), $CaCl_2$. The $2H_2O$ (0.036), Na_2CO_3 (0.02), citric acid (0.006), ferric ammonium citrate (0.006), disodium magnesium EDTA (0.001) and trace metal solution 1.0 mL L^{-1} comprising of H_3BO_3, $MnCl_2$. $4H_2O$ and Co $(NO_3)_2$. The $6H_2O$, $ZnSO_4.7H_2O$, $Na_2MO_4.2H_2O$ and $CuSO_4.5H_2O$ were composed for the growth of cyanobacteria and adjusted to a pH of 7.4. The medium was prepared according to the methods of 33, 34.

Enumeration of cyanobacteria: Ten brackish water and sediment samples collected from crude oil Bodo creek in Rivers State. The water sample was shaken to suspend sediment and then 1 mL of the raw water sample was removed and diluted into 99 mL of sterile distilled water which was serially diluted and 1 mL removed from the third dilution and plated on BG-11 medium solidified with agar and treated with ciprofloxacin and nystatin in duplicate plates. One gram of the soil sediment sample was dissolved in 9 mL of distilled water and filtered using Whatman No. 1 filter paper.

The filtrate was serially diluted and same procedure followed as in water. An aliquot of cyanobacteria was prepared using different medium-water volume ratio of 90:10, 80:20, 70:30, 60:40 and 50:50 mL and vice-versa and incubated in a cotton wool corked Erlenmeyer flask for 14 days and exposed to natural sunlight for 12 h and darkness for 12 h under ambient temperature. This was monitored and shaken twice daily to avoid sedimentation of the nutrients from the BG-11 medium.

Determination of cyanobacteria concentration: A bloom aliquot of cyanobacteria was prepared from water and filtrate from sediment samples and 1, 2, 3, 4, 5 and 10 mL was removed and inoculated into fresh growth media and was used to monitor growth for 14 days as cell density using spectrophotometer (spectronic 721 model) at 600 nm.

The spectrophotometer was set at 600 nm and 1 mL of the blank (sterile un-inoculated media) was transferred into a labelled polystyrene cavetti using sterile technique to blank the spectrophotometer. Each culture was transferred (using a sterile pipette) into labelled cavetti and the optical density reading was recorded from the spectrophotometer.

Cell counting using improved neubauer haemocytometer: The cyanobacteria concentration in the mixed culture was counted using the improved Neubauer Haemocytometer counting chamber.

A clean Neubauer cover glass was attached to the counting chamber by pressing it carefully in place. After ensuring that the cover glass was properly attached, a coloured (Newton's) ring appears between the two glass surfaces. The sample was collected using pasteur pipette and allowed to run gently through the leading edge of the cover slip into the chamber until it was filled, the counting chamber was then allowed to stand on the bench for two minutes before counting using the Leitz light microscope and examined using 10X objective for distribution of cell and refocused at 40X objective before counting the cells in the Center large square. Algal cells can be counted by using Eq. 1:

$$\text{Algal cell (mL}^{-1}) = \frac{\text{Average cell count}}{\text{Area} \times \text{depth}} \times 1000 \tag{1}$$

DNA isolation and PCR amplification of 16S rRNA for cyanobacteria: Isolation of total DNA from cyanobacterial aliquot was carried out by the method described by Tang *et al.* (2012) and Salleh *et al.* (2003).

The DNA obtained was passed through a spin column containing sepharose 43 for salt removal and humic acids. The primers CYA 106F (CGCACGGGTGAGTAACGCGTGA) and CYA 359F (GGGGAATYTTCCGCAATGGG) with a 40 nucleotide GC clamp (5' CGCCCGCCGCGCCCCGCGCCGGTCCCGCCGCCCCCGCCCG3') on the 5' end forward primer and CYA 781R (equimolar mixture of CYA781Ra (GACTACTGGGGTATCTAATCCCATT) and CYA 781Rb (GACTACAGGGGTATCTAATCCCTTT) reverse primers for amplification of a segment of cyanobacterial 16S rRNA gene were synthesized. A semi nested PCR reaction was carried out with the first reaction using primers CYA 106F and CYA 781R and followed by a reaction with primers CYA 359F and CYA781R. The PCR was carried out in a 25 µL final volume of reaction mixture containing 100 ng of DNA 2.5 µL of 10x PCR buffer with 200 µg bovine serum albumin (nuclease free) and 0.2 µ Taq DNA polymerase (BanngaloreGenei, India) in a 1 cycler (BioRad, USA). The thermal cycling profile was thus; initial denaturation for 3 min at 94°C followed by 35 amplification cycles each consisting of 1.5 min denaturation at 94°C, 1 min annealing at 59°C and a 2 min elongation at 72°C with a final 5 min elongation at 72°C.

The amplicon was further purified before the sequencing using 2 M sodium acetate wash techniques. To about 10 µL of the PCR product, add 1 µL 2 M NaAct, pH 5.2, followed by 20 µL absolute ethanol, keep at -20°C for 1 h, spin at 10,000 rpm for 10 min, then wash with 70% ethanol and air dried. Resuspend in 5 µL sterile distilled water and kept at 4°C for sequencing (Ichor *et al.*, 2014).

Approximately 500 bp long DNA derived amplification products were analyzed in parallel in DGGE buffers and gels were prepared according to the manufacturer's instructions for the Dcode™ Universal Mutation Detection system (Bio Rad). A peristaltic pump system together with the Gradient Maker (Amersham Pharmacia Biotech) were used for casting of the gels. The PCR products were separated in 6% polyacrylamide gels 0.5X TAE (20 mM Tris-acetate, 10 mM Na$_2$EDTA) with a denaturing gradient from 35-55% denaturants (urea and formamide). The DGGE gels were polymerized overnight. Electrophoresis was performed for 4.5 h at a constant voltage of 150 V and a temperature of 60°C in the Dcode™ apparatus containing a magnetic stirrer bar. After electrophoresis, the gels were stained for 40 min with Gelstar nucleic recorded under UV-light using Kodak 1D image analysis software.

Gene sequencing and analysis: The cocktail mix is a combination of 9 µL of Hi-Di Formamide with 1 µL of purified sequence making a total of 10 µL. The samples were loaded on the ABI machine and the data in form of A, C, T and G, was released. Sequence results obtained from above were compared with known sequences in the GenBank using the basic local alignment search tool of the national Center for biotechnology information. Species were identified based on the percentage similarity with the known species sequences in the data base (Ichor *et al*., 2014).

Preparation of inoculum and biodegradation experiment: A bloom culture of cyanobacterial aliquot using BGII medium formulations was also prepared by transferring 1 mL of each viable culture in to 400 mL of the medium and incubated for 14 days under natural sunlight for 12 h and darkness for 12 h.

Brackish water samples labelled CB were prepared by aseptically transferring 200 mL cyanobacteria aliquot into 500 mL of sterile distilled water and standardized using 0.5 M McFarland solution. The inoculum size used for the treatment was 100 mL of standardized aliquot. The water containers for the experimental set up were each filled with 11 L of water and labelled CB for treatment with cyanobacteria and C for the control and contaminated with 32300 ppm of sterile bony light crude oil sample obtained from shell petroleum development company. Water sample CB was treated with 0.25 mg mL^{-1} of ciprofloxacin and nystatin to prevent growth of bacteria and fungi in the set-up, while the control was left untreated. The containers for the experiment were washed with detergents and rinsed severally with distilled water prior to the experimental set up.

Biodegradation activities were monitored for a period of 49 days for petroleum hydrocarbon degradation using gas chromatography (GC-FID).

RESULTS
Total Petroleum Hydrocarbon (TPH) biodegradation experiment: Cyanobacteria cells were obtained using different water samples to BG II broth ratio as presented in Table 1.

In an attempt to determine the capability of isolated aerobic heterotrophic bacteria from Bodo creek in biodegradation of total petroleum hydrocarbon, water samples from a section of the uncontaminated Bodo creek adjudged to be unpolluted by crude oil as observed in our preliminary result of the gas chromatography conducted on it and physical observation were contaminated with known volume of bony light crude oil. The TPH present were C-9,C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23, C-24, C-26, C-30 and C-32 for the treatment on day 0 (9). The total amount of TPH after contamination of water with crude oil showed that the
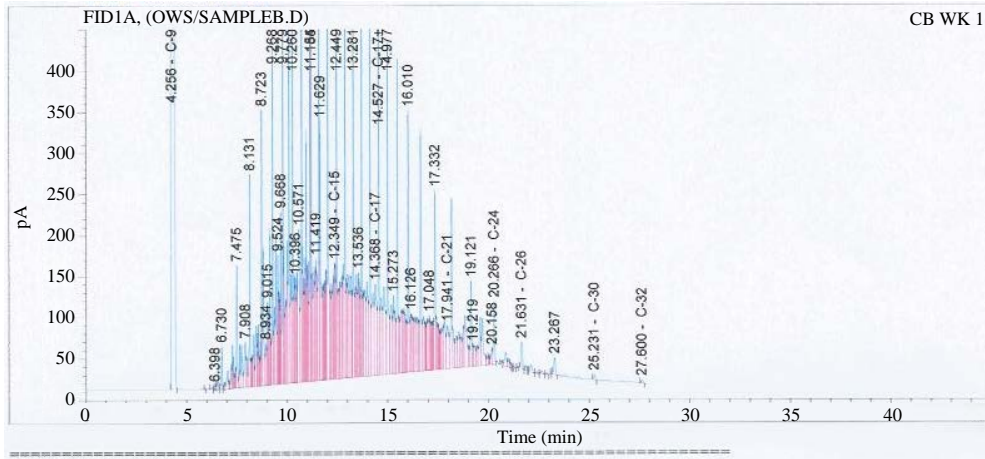
Fig. 1: Chromatogram of crude oil contaminated water inoculated with cyanobacteria on day 0
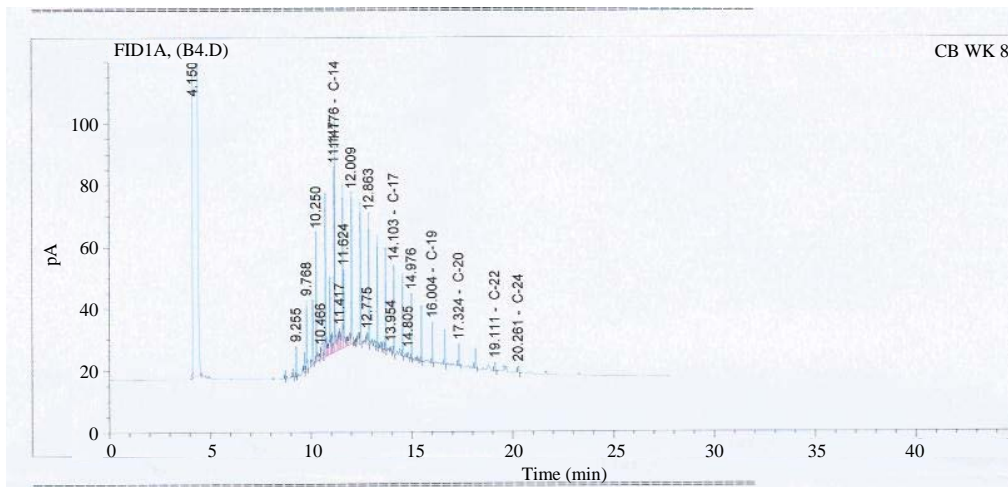

Fig. 2: Chromatogram of crude oil contaminated water treated with cyanobacteria on day 49

Table 1: Cell density (OD 600 nm) of different aliquot from bloom algal culture from water samples (water-medium rate)

Time (h)	90:10	80:20	70:30	60.40	50:50
0	0.021	0.025	0.022	0.026	0.028
24	0.08	0.15	0.18	0.19	0.23
48	0.12	0.19	0.26	0.28	0.32
72	0.16	0.22	0.29	0.32	0.41
96	0.19	0.28	0.33	0.38	0.45
120	0.22	0.32	0.34	0.43	0.49
144	0.26	0.38	0.41	0.46	0.58
168	0.36	0.47	0.49	0.51	0.65
720	1.4	2.52	3.72	3.82	5.84

treatment had TPH of 29882 mg L^{-1} for the first day and reduced significantly to 753.7 mg L^{-1} on the first and last day of sampling. The chromatograms in Fig. 1 and 2 represent the quantity of TPH for the first and last day of sampling, respectively. It was observed that the consortium of
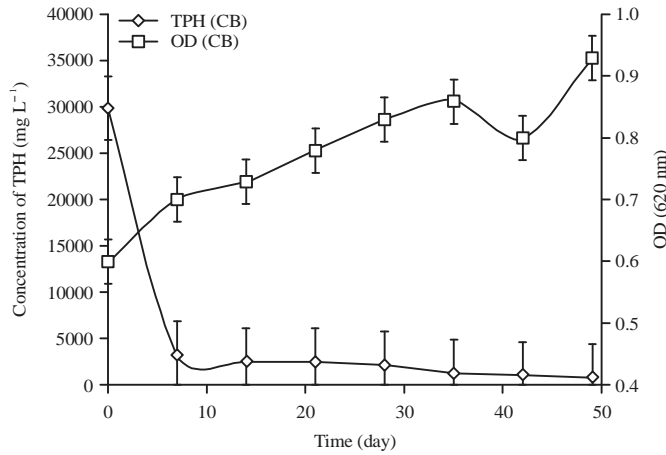
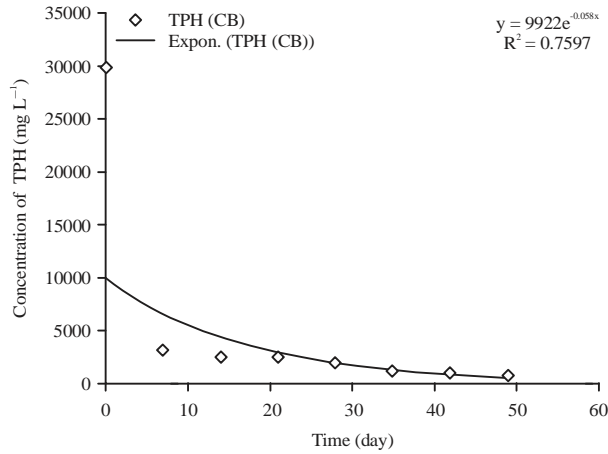Fig. 3: Total petroleum hydrocarbon degradation by cyanobacteria

Fig. 4: Model for total petroleum hydrocarbon degradation by CB

cyanobacteria degraded TPH with corresponding exponential growth throughout the period of the experiment (Fig. 3). Figure 4 shows modelling of TPH degradation by cyanobacteria using the Trend line method of Microsoft Excel 2010. It was observed that the consortium of cyanobacteria was able to degrade TPH progressively. The curves indicate the presence of volatile compounds in the contaminated samples because of the observed initial sharp decrease in TPH in the treatment. The coefficient of determination (R^2) is an indication of how well the model can be used to predict TPH degradation with time for the treatment options. The CB coefficient of determination (R^2) for the quantity of TPH left per time using the Eq. 2:

$$TPH\ (Y) = 9922e^{-0.058x} \tag{2}$$

where, x is the time. The coefficient of determination of values (R^2) (goodness of fit = 0.7597) which implies that if the experiment is repeated, there is possibility of obtaining the same result at 75.97% confidence level for CB and can be used to determine the quantity of TPH left.
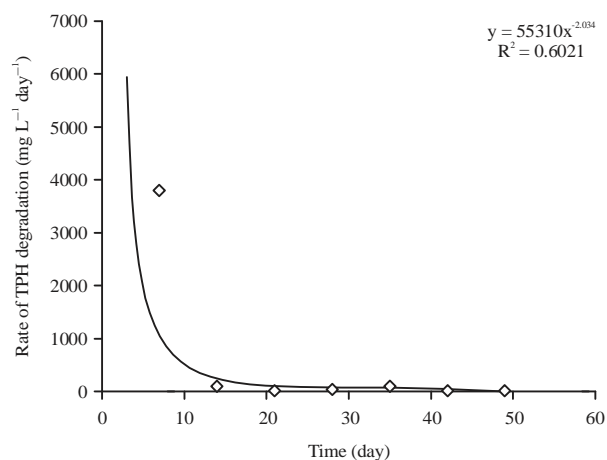
Fig. 5: Rate of total petroleum hydrocarbon biodegradation by CB

Table 2: Cyanobacteria isolated from brackish water of Bodo creek

Microorganisms	Similarity (%)	Strains	Accession No.
Microleus sp.	81	MMG-10	KF157400.1
Oscillatoriales cyanobacterium	83	EcFYyyy400	KC163194.1
Phormidium sp.	83	195A12	EU282429.1
Synechococcus sp.	84	MI0607D	GQ130146.1
Microcystis elabens	79	NIES4279	U40335.1
Anabaena cycadae	81	MMG-11	KF15740.1
Pseudanabaena minima	83	GSE PSE20-05C	HQ132935.1
Leptolynqbya sp.	83	KIOST-1	JX401929.1
Microcystis holsatica	79	NIES4379	U40336.1
Chlorella kessleri	100	SAG211-11	
Phormidium faveolaurum	100	ISC64	
Filamentous cyanobacterium	83	N10-SD2	KF793929.1

The rate of TPH degradation was monitored for all the treatments in the experiment and was high at the beginning of the experiment but decreased with time due to substrate exhaustion resulting from substrate utilization or degradation by microorganisms involved. A negative relationship was observed between the rate of TPH degradation and time (days), r value of -0.78. Figure 5 shows the daily rate of TPH degradation by CB per each day, using the Eq. 3:

$$Y \text{ (rate of TPH degradation)} = 55310x^{-2.034} \tag{3}$$

where, x is time. The 16S rRNA sequencing for identification of the isolates generated sequences ranging from <600 bp and a 250 bp size PCR amplified fragment. The nucleotide sequence of the 16S rRNA gene was compared with published 16S rRNA sequences using BLAST search at the data base of National Center for Biotechnology Information (NCBI) (Table 2).

DISCUSSION

The capability of cyanobacteria to degrade compounds including petroleum hydrocarbons has been reported by other researchers and evidence abound on the fact that microbial communities dominated by cyanobacteria are actively involved in oil degradation (Grotzschel *et al*., 2002; Sanchez *et al*., 2005; Abed *et al*., 2000). The growth of cyanobacteria after oil spills into the Arabian Gulf forming heavy thick mats gave the impression that cyanobacteria possess the potential to

degrade oil components (Al-Hasan *et al.*, 1994; Sorkhoh *et al.*, 1992). In this present study, BGII broth was prepared and treated with ciprofloxacin and nystatin which our trial experiment showed no bacterial and fungal growth on solidified BGII using agar inoculated with the raw water samples from crude oil contaminated Bodo creek. The water and filtered sediment sample inoculated in the BGII both bloomed and formed axenic cultures of cyanobacteria consortium and were grown on solid BGII and identified using the 16S rRNA gene sequence analysis. The cyanobacteria identified and their percentage similarities were C*hlorellakessleri* SAG 211-11gx (100%), *Anabaena cycadae* MMG-11 (81%), *Phormidium faveolaurum* strain 1SC 64 (100%) etc. (Table 2).

Previous studies that focused on the capacity of cyanobacteria to degrade hydrocarbon reported on similar cyanobacteria found in this study (Cerniglia *et al.*, 1980; Narro *et al.*, 1992; Raghukumar *et al.*, 2001; Al-Hasan *et al.*, 1998; Diestra *et al.*, 2007; Lei *et al.*, 2002).

Total petroleum hydrocarbon degradation as monitored in this studyprovides evidence that the consortium of cyanobacteria isolated from thecrude oil contaminated Bodo creek possess an inherent capability for biodegradation of petroleum asmonitored using GC-FID. This is in agreement with the report of other researchers who carried out similar studies in separate studies across the globe. For instance, (Raghukumar *et al.*, 2001) monitored removal of approximately 55%of total fractions of crude oil comprising of 50% aliphatics, 31% waxes and bitumen, 14% aromatics and 5% polar compounds by mixed cultures of three cyanobacterial species which disappeared within 10 days measured using gravimetric and gas chromatographic methods. Previous studies in literature monitored degradation of petroleum hydrocarbons by the cyanobacteria *Nostoc punctiforme* and *Spirulina platensis* and reported total removal of decane, pentacosane, hexacosane, octacosane and nonacosane using gas chromatography mass spectroscopy. Species of *Oscillatoria salina*, *Plectonema terebans*, *Aphanocapsa* sp. and *Synechococcus* sp., grew as mats in aquatic environments and have been used successfully in bioremediation of oil spills (Raghukumar *et al.*, 2001; Radwan and Al-Hasan, 2001; Cohen, 2002). Oil contaminated soil was also successfully remediated using naturally occurring cyanobacterial-bacterial association (Sorkhoh *et al.*, 1995). El-Bestawy *et al.* (2007) tested biodegradation of a well-known chemically and biologically recalcitrant and slow degrading Lindane using some isolated cyanobacterial species with high prove of efficiency in degrading the pesticide at a very fast rate as well as demonstrating high level of resistance against its toxicity.

The reduction of TPH in this present study was observed to be faster during the first seven days (Fig. 3) implying that volatile components of the crude oil had evaporated. The TPH loss was statistically significant ($p<0.05$) with time throughout the experimental period using one way analysis ofvariance. The growth of cyanobacteria increasedprogressively with decrease in TPH throughout the 49 days periodmonitored and could be attributedto the presence of optimum environmental factors and limitingnutrients which were adjusted to mimic the natural environmentalconditions from which cyanobacteria were isolated and othermetabolites suspected to have been generated in the course of biodegradation. A negative correlation between growth of cyanobacteria and TPH loss was observed for both the treatment and the control ($p<0.05$) implying that cyanobacterial growth increased with decreasing quantity of TPH for the treatment and the control. Ichor *et al.* (2014) and Latha and Kalaivani (2012) reported a correlation between increased oil degradation to an increase in cell number of bacterial indicating that the isolates were responsible for the degradation which corroborates the result of our present finding. The rate of biodegradation has been widely reported to be affected by a number of factors. This study considered these factors and adjusted our set up to reflect the factors at the site where the pollution

occurred and samples were obtained in order to ascertain near accuracy the activities of the autochthonous cyanobacteria at the pollution site.

Treatment of cyanobacteria medium with ciprofloxacin and nystatin cured it of any associated microorganisms (bacteria and fungi) but did not hinder their growth as evidenced by their bloom measured by optical density. The values for cyanobacteria cell counts using improved Neubauer Haemocytometer progressively peaked with time throughout the culture and experimental period. Though cyanobacteria may require aerobic heterotrophic bacteria for their growth, it is not however a limiting factor and thus not difficult growing them in axenic cultures as reported by McGenity *et al.* (2012) and Abed and Koster (2005).

The result established the fact that cyanobacteria isolated from crude oil polluted Bodo creek though photosynthetic are capable of utilizing crude oil as a source of carbon and energy. Isolated species investigated for biodegradation in this present study are highly recommended for beneficial bioremediation applications for *in situ* and *ex situ* removal of petroleum hydrocarbons in the crude oil polluted and devastated Niger Delta area of Nigeria since they are resident flora in this region and have already been adapted to the site conditions of crude oil pollution among other factors.

Cyanobacteria are photosynthetic and are believed to acquire carbon as a source of energy from atmospheric CO_2 and have been seen to degrade hydrocarbons. An investigation into whether it utilizes the carbon in the petroleum hydrocarbons and when, should be undertaken. Studies using DNA based molecular identification and phylogenetic analysis to establish the community structure of cyanobacterial populations during biodegradation of petroleum hydrocarbons contaminated brackish waters should be undertaken.

Further research on biostimulation and bioaugmentation should be carried out in order to establish the best and efficient methods of optimizing the degradation potentials of the isolates.

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