International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 7, November-December 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Hydrocarbon Biodegradation Potential of Cyanobacteria in Oil Polluted Soil

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ABSTRACT

Petroleum hydrocarbon is one of the most common environmental pollutants in Nigeria and other countries. Its debilitating effects on arable lands and water bodies have far reaching consequences to agriculture and aquatic life. Several bioremediation strategies have been developed over the years in a bid to manage this type of pollution. Cyanobacteria are a group of microorganisms possessing the potential to be harnessed as bioremediation alternatives. This study sought to conduct bioremediation of crude oil polluted oil usung cyanobacteria. Organisms were isolated from crude oil polluted soil and were identified using molecular typing. A 200 ml portion of broth cultures of organisms was used singly and in consortium in different set ups to bio-remedaite the polluted soil samples. Gas chromatography was used to monitor the residual total petroleum hydrocarbon (TPH) in each set up for a period of thirty days. *Microcystisholsatica, Chlorella kessleri*and *Anabaena cicadae* were identified from polluted soil samples. Chlorella kessleri gave a 94.10% TPH loss, while *Anabaena cicadae* gave 91.10% and their co-culture set up gave a 95% TPH loss and the control experiment had 2.10% TPH loss. Temperature of the reacting systems was mesophilic and the pH was at weak acid range. This study thus has shown that cyanoremediation of petroleum hydrocarbon pollution of soil is possible and efficient and should be adopted more often

KEYWORDS: *Hydrocarbon, Biodegradation, Pollution, Cyanobacteria, Soil*

How to cite this paper: Agu, K. C. | Orji, M. U. | Ikele, M. O. | Uwanta, L. I. | Onyeneho, V. I "Hydrocarbon Biodegradation Potential of Cyanobacteria in Oil Polluted Soil" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-7, December 2022, pp.733-737, URL: www.ijtsrd.com/papers/ijtsrd52397.pdf

IJTSRD52397

1. INTRODUCTION

1.1. Background of the Study

Crude oil is currently Nigeria's and of course, the world's main energy source (Aguet al., 2015. Petroleum is simply amixture of crude oil, condensate and natural gas. Crudeoil is a complex mix of different molecular weight hydrocarbons made up of hydrogen and carbon in the ratio of 2:1. It is also composed of approximately 3% (v/v) nitrogen, oxygen and sulphur; trace amounts of phosphorus and heavy metals viz vanadium and nickel (Okaforet al., 2016; Anaukwuet al., 2016; Hassanshahian and Cappello, 2019). There exist 4 classes of hydrocarbons in crude oil ***namely:*** the resins and the as phaltenes; the more polar, non-hydrocarbon components and the saturated hydrocarbons and the aromatics. In Nigeria, oil producing areas particularly the Niger Delta have borne the brunt of **oil spill**age on both the aquatic and terrestrial environments for more than 5 decades of crude oil exploration and production (Kadafa, 2012; Agu and Odibo, 2021). The Niger Delta is among the top 10 most important wetlands and marine ecosystems on earth, whose ecosystem has been brutally devastated by petroleum contamination due largely to poor petroleum exploration (Mbachu*et al.,* 2014).

In aquatic and terrestrial ecosystems, the biodegradation of crude oil and other petroleum mixtures has been believed to be brought about by the actions of bacterial and fungal populations and as such little attention has been paid to cyanobacteria, which possess better capacities of withstanding adverse environmental conditions than most bacteria and fungi (Ichor *et al.,* 2016). Cyanobacteria are oxygen evolving photosynthetic unicellular organisms

that are thrive in inhospitable environments (Thajuddin and Subramanian, 2005). Cyanobacteria, also called blue green algae are remarkably genetically diverse, inhabiting a wide variety of environments across the globe (Kulasooriya, 2011). There is no uncertainty however, that cyanobacteria perform vital roles in microbial mat by creating oxygen gradients and providing nutrients for heterotrophic bacteria. A host of cyanobacteria have been demonstrated to be able to degrade hydrocarbons such as *Oscillatoria*sp. and *Agmenellumquaduplicatum* showed the capacity to oxidize naphthalene to 1-naphthol; *Microleuschthonoplastes* and *Phormidium corium* ingested and oxidized large amounts of n-alkanes; *Picocyanobacteria* from the Arabian Gulf have also been shown to accumulate enough hydrocarbons (Ichor *et al.,* 2016).

2. Methods

2.1. Study Area

Soil Samples were collected from Addas Petroleum Development in Izombe community, Oguta L.G.A, Imo State Nigeria. Izombe community covers 391km in Imo State, South-East Nigeria.

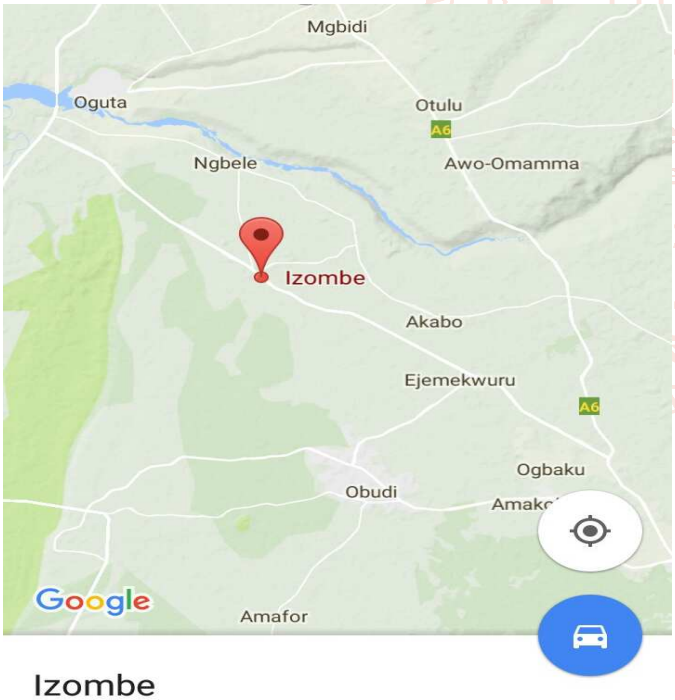

Fig 1: Location of Izombe Community on the map

2.2. Soil Sample Collection

Two sets of soil samples were collected (Sample A and B). For Sample A, 1.0 kg each of the oil polluted soil was collected from ten random points on the site at a depth of 15cm to give a total of 10kg of soil sample for microbial isolation. For Sample B, 2.0 kg each of the unpolluted soil was collected from ten random points on the site at a depth of 15cm to give a total of 20 kg of soil sample.

2.3. Isolation of Cyanobacteria

The isolation of cyanobacteria was done using the method adopted from Ichor *et al.* (2016), using BG-13 medium. A 2.0g portion of the soil sample was inoculated into the BG-13 medium in a flask and placed near the window for sunlight in order to aid photosynthesis. The culture was incubated under room temperature, night and day until growth was observed within two weeks. Nystatin, cycloheximide and chloramphenicol were added to the culture medium during preparation at a concentration of 0.1mg/ml to prevent fungal and bacterial contamination.

The cyanobacterial colonies growing on the BG-13 medium were picked by a sterile wire loop and was transferred aseptically into 50 ml of sterile BG-13 broth. The pure cultures were incubated for 2 to 4 weeks under the conditions described previously in order to obtain sufficient cyanobacterial biomass, and then 400µl of a sterile nutrient solution (SNS) consisting of 2.5% (wt/vol) sucrose, 0.5% (wt/vol) yeast extract, and 0.5% (wt/vol) Bacto-Peptone was added along with 400 RI of sterile antibiotic solution [Aqueous stock solutions (0.5% [wt/vol]) of cefoxitin, ampicillin, penicillin G, imipenem, andcycloheximide (1.25% [wt/vol]) (100 µg/ml). Cultures were incubated for 18 to 24 h. After incubation, the cyanobacteria were harvested by centrifugation at 17,000 x g for 15 min at 25°C. It was then pipetted and plated onto BG-13 agar containing nystatin and cycloheximide. The plates were incubated for 2 to 4 weeks and observed at weekly intervals for the growth of cyanobacteria. Purified colonies of cyanobacteria were picked and transferred to plates of BG-13 agar.

2.4. Purity Check of the Cyanobacterial Organisms

The purity of isolates were observed by light microscopy and by inoculating cyanobacterial growth into BG-13 broth supplemented with 0.01% (wt/vol) (each) glucose, yeast extract, and Bacto-Peptone and into GNB broth medium, which consisted of 1.0% (wt/vol), glucose and 0.8% (wt/vol) nutrient broth. Broth cultures were incubated at 25°C with shaking at 180 to 200 rpm under ambient atmosphere and statically under anaerobic conditions in an anaerobic jar. The culture was judged axenic as there was no growth of heterotrophic bacteria.

2.5. Molecular Analysis

DNA Extraction of organisms was performed at the Molecular Biology and Biotechnology Laboratory, NnamdiAzikiwe University, Awka and sent to Inqaba Biotech South Africa for sequencing and typing.

2.6. Preparation and Development of Inoculum

A loop full of the isolated cyanobacteria was transferred onto a 10 ml of BG-13 broth medium formulations and incubated for 14 days under natural sunlight for 12 hours to allow the cyanobacteria to multiply. After multiplication in the broth, it was then aseptically transferred and scale-up from 20 ml to 50 ml to 100 ml to 200 ml and 400 ml.

2.7. Biodegradation Set-up

The soil sample was autoclaved, divided into four parts of 5kg each and was poured into sterile transparent bucket labeled A, B, C, and D.A 0.5 L quantity of the escravos crude oil was added to 5kg of soil in each setup. To prevent any fungal, bacterial and cyanobacterial activity, 0.25 g/kg each of nystatin, streptomycin, $CuSO_4$ was added to the respective buckets. To SETUP A, 200 ml of culture suspension of *Chlorella kessleri* was inoculated; to SETUP B, 200 ml of culture suspension of *Anabaena cycadae* was inoculated; to SETUP C, 200 ml each of culture suspension the two cyanobacterial organisms were inoculated while SETUP D served as the control where no organism was inoculated. The setups were incubated at room temperature near a transparent glass window to allow rays of sunlight to reach the setups and were monitored throughout the experimental period of 30 days.

Evaluation of Process Parameters during Bioremediation

Temperature, pH and moisture content were monitored during the bioremediation period. Temperature readings were within 30°C and 32.5°C as shown in Table 2. Moisture content of the set ups were relatively lower when compared to the control experiment (Table 3) while pH of the system ranged between 5.8 to 6.2 as shown in Table 4.

Moisture Content, pH and Temperature of the biodegradation microcosms were also determined.

2.8. Data Analyses

The data generated were analysed for mean and also with analysis of variance (ANOVA) at 955 confidence interval. Mean partitioning was done using Duncan multiple range test.

Results

Isolation and Identification of Cyanobacterial Organisms

Three cyanobacterial organisms were identified from the crude oil contaminated soil using molecular typing, and they are: *Microcystisholsatica, Chlorella kessleri*and *Anabaena cicadae.*

Evaluation of Hydrocarbon Degradation Potentials of Isolates

Chlorella kessleri bioremediation set up A had a lower residual total petroleum hydrocarbon value than *Anabaena cycadae* set up B over the monitoring period, with a total PH percentage loss of 94% and 91% respectively. The best residual TPH value which reflects the level of bioremediation efficiency of the isolates was 194.09^{a}±0.00 ug/ml with a percentage TPH loss of 95.30%, obtained from set up C which is a bi-culture cocktail of the cyanobacterial organisms as shown in Table 1.

Table 1: Total Petroleum Hydrocarbon Contents of the Bioremediation Set-up

Time (weeks)	Setup A	Setup B	Setup C	Setup D
0	4132.30^{a}±0.10	4132.30^{a}±0.10	4132.30^{a}±0.10	4132.30^{a}±0.10
7	1052.98^{b}±0.01	1258.09^{c}±0.00	741.58^{a}±0.01	4112.15^{d}±0.01
14	565.36^{b}±0.02	984.50^{c}±0.10	387.72^{a}±0.02	4105.45^{d}±0.01
30	241.93^{b}±0.10	367.54^{c}±0.01	194.09^{a}±0.00	4046.92^{d}±0.01
% TPH Loss	94.10^{d}±0.01	91.10^{b}±0.10	95.30^{c}±0.01	2.10^{a}±0.01

Mean values along the same row with different affixes are significantly different ($p<0.05$); A: *Chlorella* bioremediation group; B: *Anabaena* bioremediation group; C: Cyanobacteria co-culture bioremediation group; D: control group.

Table 2: Temperature Changes of the Bio-remediation Set-up

Time (months)	Setup A	Setup B	Setup C	Setup D
0	32.00^{a}±1.00	32.00^{a}±1.00	32.00^{a}±1.00	32.00^{a}±1.00
7	31.00^{a}±1.00	31.60^{bc}±0.10	30.40^{b}±0.02	30.70^{c}±0.10
14	32.00^{b}±1.00	30.80^{a}±0.10	32.70^{c}±0.10	32.35^{c}±0.01
30	32.30^{c}±0.20	32.00^{b}±1.00	31.00^{a}±1.00	31.65^{d}±0.01

Mean values along the same row with different affixes are significantly different ($p<0.05$); A: *Chlorella* bioremediation group; B: *Anabaena* bioremediation group; C: Cyanobacteria co-culture bioremediation group; D: control group.

Table 3: Moisture content (%) of the Bio-remediation Set up

Time (Days)	Setup A	Setup B	Setup C	Setup D
0	6.20^{a}±0.10	6.20^{a}±0.10	6.20^{a}±0.10	6.20^{a}±0.10
7	8.95^{a}±0.01	10.20^{d}±0.10	9.10^{b}±0.10	9.93^{c}±0.01
14	8.05^{c}±0.01	9.40^{d}±0.10	7.70^{a}±0.10	7.88^{b}±0.01
30	14.10^{d}±0.10	11.00^{a}±0.50	13.80^{b}±0.10	1395^{c}±0.01

Mean values along the same row with different affixes are significantly different ($p<0.05$); A: *Chlorella* bioremediation group; B: *Anabaena* bioremediation group; C: Cyanobacteria co-culture bioremediation group; D: control group.

Table 4: pH Changes of the Bio-remediation Set-up

Time (Days)	Setup A	Setup B	Setup C	Setup D
0	6.17^{a}±0.01	6.17^{a}±0.01	6.17^{a}±0.01	6.17^{a}±0.01
7	5.98^{a}±0.01	6.10^{b}±0.10	5.94^{a}±0.01	5.96^{a}±0.01
14	5.93^{a}±0.01	6.07^{b}±0.01	5.93^{a}±0.01	5.89^{ab}±0.00
30	5.85^{a}±0.01	6.04^{c}±0.01	5.92^{b}±0.01	5.85^{a}±0.01

Mean values along the same row with different affixes are significantly different ($p<0.05$); A: *Chlorella* bioremediation group; B: *Anabaena* bioremediation group; C: Cyanobacteria co-culture bioremediation group; D: control group.

Discussion

Cyanoremediation is a sustainable and eco-friendly bioremediation alternative. It consists of blue-green algae driving the bioremediation process, which is a welcomed idea because the world is going green and the way of renewable energy sources. The use of fungi and bacteria for hydrocarbon bioremediation has been in the research space for some decades, however, the diversion to the use of cyanobacteria holds better benefits like removal of wide range of organic contaminants, reduction in the contribution of greenhouse gases and consequent global warming, they have lipid accumulation efficiency and are readily available in the environment where they grow with little or no stringent culture conditions like bacteria (Gupta *et al*., 2012; Touliabah*et al*., 2022).

Cyanobacteria isolated in this study are *Microcystis holsatica, Chlorella kessleri* and *Anabaena cicadae* and this varies from the reports of El-Sheekh and Hamouda (2013) who isolated *Nostocpunctiforme* and *Spirulina platensis*. This finding also corresponds partly with that of Aldaby *et al*. (2018) that reported the isolation of *Chlorella* from hydrocarbon polluted soil.The bioremediation experiment revealed that these cyanobacteria had the capacity to degrade petroleum hydrocarbon – a role attributed to the action of enzymes produced by these organisms

(Aldabyand Mawad, 2018). It was also observed that temperature process of the bioremediation set up was mesophilic and it corresponds with the report of Ghasemi*et al*. (2011). This present study showed that bioremediation process driven by co-cultures of the two choice cyanobacterial isolates gave 95% total petroleum hydrocarbon loss within a period of thirty days, which serve as an indication for their suitability in hydrocarbon bioremediation process. This field of bioremediation is not yet well tapped into, but possesses the possible green energy alternative the field of microbiology can contribute to the world's discourse on global warming, climate change, renewable energy and green energy options.

Conclusion

It has been shown through this study that cyanobacteria are potent microbial options for the bioremediation of crude oil polluted soil environment. This is in addition to other pollutants which they have been reported to also degrade. It therefore becomes imperative that to manage the environmental damage caused by oil spillage, cyanobacteria are viable alternatives. They are sustainable, renewable, easy to cultivate and contain enzyme complexes that aid them use petroleum hydrocarbons as sole carbon source.

Acknowledgement

I want to acknowledge in a very special way the efforts of Tertiary Education Trust Fund (TETFund) National and that of Nnamdi Azikiwe University for providing us with the Institution Based Research (IBR) Grant for the execution of this research; as well as the Vice Chancellor of Nnamdi Azikiwe University, for providing an enabling environment for this research to be conducted.

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