Original Research Article

ISOLATION AND IDENTIFICATION OF MICROORGANISMS ASSOCIATED WITH BIOREMEDIATION OF OIL SPILLED SITE IN BODO WEST, RIVERS STATE, NIGERIA.

Abstract

The samples collected from an oil spilled sites in Bodo West in Gokana Local Government of Rivers State in Nigeria were isolated to identify microorganisms associated with bioremediation. The population of about 311 different forming colonies were recorded in the study area; out of which 18 distinctive colonies were identified based on their morphological observation. From the selected isolates, 10 of them were assumed to be degraders because they form maximum clear zones on the mineral salt media. The results of the analysis show that notable number of microorganism of which seven bacteria and seven fungi were isolated and identified. The bacteria are *Micrococcus Luteus, Streptococcus Lactic, Streptococcus Epidemidis, Streptococcus Faecalis, Clostridium Sprogenes, Aerococcus Viridems, and Bacillus Anthracis.* The fungi are *Articulosspara inflate, Dendospora Erecta, Aspergillus Niger, Liodioderium Species, Geotichrum Albdum, Aspergillus Funigatus and Sreptothric Atrax.* On the strength of the result, it is inferred that microorganisms are associated with bioremediation and can be used for environmental and petroleum cleanup exercise in an oil spilled site.

Keywords: microorganisms, biodegradation, bioremediation, hydrocarbons, oil spilled, isolation, fungi and bacteria.

1. Introduction

Petroleum exploration is a lucrative business especially in Nigeria [7,15,34]. Nigeria since the discovery of oil has survived on the proceeds from oil production, as capital projects and paying of workers' salaries are dependent on income generated from the oil business [12,14,36]. Though, there had been calls from different quarters for diversification of the economy from the solo means of petroleum exploration into other sectors like agriculture, commerce and manufacturing [14]; however, the current gains for petroleum resources has overshadowed government interest in other areas of the economy [13,32].

Petroleum exploration involves a complex process; from drilling, refining to the distribution of the products to the different marketers and end users [13,36]. The processes have its own associated environmental problems like oil spills on a large scale on the land, sea or river and massive air pollution has been reported [1,8,9,11,12,16,17,18,21,29,30]. The government had in the past carried out environmental programmes to educate the people on the consequences of pollution [22,32,35]; but the people have always these rejected government programmes due to their non-participation in the decision making the process [31]. Such agitations by the people in the local communities have always resulted into violent conflicts [10,19,23,24,25].

Hydrocarbon contamination of the environment has not only destroyed the ecosystem but has also resulted in several health challenges and deaths [33]. Thus, there had been calls for remediation of polluted land in the Niger Delta [31]. Mechanical and chemical methods are generally used to remove hydrocarbons from contaminated sites [26-28]. These methods have limited effectiveness and can be expensive; so bioremediation is a promising technology for the treatment of these contaminated sites since it is cost effective and will lead to complete mineralisation [26-28]. The process of

bioremediation is simply the used of microorganisms to remove pollutants from the polluted environment through the establishment and maintenance of a condition that favours oil biodegradation rates in the contaminated environment [26-28,2-5].

Bioremediation becomes a process of interest in the petroleum industry due to the success in the cleanup of the oil tanker Exxon Valdez of oil spill of 1989 [6,26-28). Bioremediation is an attractive technology that has gained popularity in global conservation and sustainability strategies [26-28]. The interest in microbial biodegradation of pollutants has been so pronounced in recent times as there had been calls for sustainable ways of cleaning up contaminated environments [37]

2. The Study Area

The aim of this study is to isolate and identify the microorganisms that are associated with bioremediation of oil spilled site in Bodo West in Gokana Local Government Area (LGA) of Rivers State. Bodo West is a small village settlement in Gokana Local Government Area in Ogoni. Ogoni (comprise of four Local Government Areas - Gokana, Khana, Tai and Eleme) which is a superset of Bodo West lies between latitude $4^{0}05^{1}$ and $4^{0}20^{1}$ North and longitude between $7^{0}10^{1}$ and $7^{0}30^{1}$ East [36]. It is accessible by roads and footpath and some parts that are covered by thick vegetation were inaccessible.

3. Materials and Methods

Sampling and Sample size

The sampling techniques that were used for this study is a random selection. This sampling method was adopted to give each soil bacterium or fungal species a chance to be represented in the microorganism population within the study area. The population of this study identifies about 311 different colonies on the different serial dilution plating out. Out of the different colonies, 18 distinctive colonies based on morphological observation from the different locations on the dilation plate were identified to form a ratio 5.7% of the population of the study.

Isolation and Identification of Microorganism

Soil samples were collected using sterilized spatula at a tillage depth of 2cm randomly from 10 core points. For testing of the ability of isolates to degrade crude oil mineral salt media was prepared. The media for this study include Bushnell Haas, Nutrient Agar and Blood agar. The Bushnell Haas broth medium contains 2.0g of $MgSO_4$, 0.53g of KH_2PO_4, 053g of K_2HPO_4, 0.02g of $CaCl_2$, 0.63g of NH_4NO_2 and 0.05g of $FeCL_2$ (Keterazol). The Nutrient Agar contains 5g of peptide digest, 5g of yeast extract, 5g of beef extract, 5g of NaCl and 2g of Agar. The PH was adjusted to 7.2 and the media was autoclaved at $121^{o}C$ for 15 minutes. The bacteria were isolated from the soil samples by culturing them through the growth conditions of the media. 1g of well powered and sieved oil polluted soil samples were weighted and dissolved in 10ml of sterilized distilled water in in ten replicates and shaken thoroughly. Aseptically, 9ml of distilled water was pipette into ten (10) different test tubes and labelled accordingly from (10^{1} to 10^{10}). 1g of the soil sample A was weighed and transferred into the test tube labelled 10^{1} and then from 10^{1}, 1ml was pipette into 10^{2} and 10^{3} accordingly. The process was repeated at each dilution factor using a different pipette to avoid cross-contamination. The steps stated above were then repeated for the remaining soil samples and the test tubes were shook for proper homogenization. The pour plate was used for the inoculation method. Iml of the diluted sample was aseptically pipetted into the labelled petri dish plates. The dilution factor (10^{1}, 10^{4} and 10^{8}) was

used. The prepared nutrient agar media at 45^{o}C was poured into all the plates and mix properly. The plates were then placed in an incubator at 37^{o}C for one week to be incubated. The growth of the organisms was carefully observed on the plates and the distinct colonies were selected from the nutrient agar. The different colonies of different shapes, colours and sizes were selected from the different agar plates and sub cultured for more analysis as shown on Table 3.

Screening of Hydrocarbon-degrading fungi and bacteria.

To isolate the pure culture of hydrocarbon-degrading bacteria in the soil samples, each of the isolate was inoculated into newly prepared and properly sterilized Bushell Haas Broth medium that was enriched with nutrient agar. 1ml of sterilized crude oil was added as a source of carbon and subsequently, 10ml of Keterazol was also added to the Bushnell Haas medium to prevent the growth of fungi. The flask that contained was then incubated at 30^{o}C with regular shaking for two weeks. The content of the flask was then observed at a regular basis for any changes in hydrocarbon concentration, colour and optical density for the same period of two weeks. For fungi, about 5ml of selected four (4) dilution factor source was dispensed into sterile Petri dishes. Nutient agar (3.6g) was poured into 100ml distilled water; which was later transferred into a conical flask using pour plate method. The petri dish was incubated at normal room temperature for 72 hours. Every observation was recorded for proper analysis. This procedure is in line with the works of other scholars [38-41].

4. Result and Discussion

The bacteria isolates from the subculture were identified by biochemical test. Organism isolated and identified were seven fungi and seven bacteria. The bacteria isolate are clostridium sparogerms. Aerococus viridians, streptococcus Lactic, Micrococcus Lutes, Staphylococcus Lactic, Staphylococcus Epidermis, Streptococcus epidermis, Streptococcus Faecalis, Bacillus anthraces. The seven fungi isolated and identified are: *Articulospara Infalta, Dendospora erecta, Aspergillus niger, Loidioderium Species, Geotichrum albidum, Aspergillus funigatus and Streptothrix atrax*. The result is shown on Table 1 and table 2.

TABLE 1: Biochemical characterization of bacterial isolates

Catalase	-	-	-	-	-	-	-	Z+
Motility	-	-	-	-	-	-	-	-
Hydrolysis	+	+	+	+	+	+	+	+
Glucose	A	A	A	A	A	A	A	A
Lactose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
maltose	+	+	+	+	+	+	+	+
Arabinos	+	+	+	+	+	+	+	+
Coagulase	-	-	-	-	-	-	-	-
Shape	Circular	Sphere	Sphere	Sphere	Round	Round	Dombel	Round
Edge	Dented	Enteric	Dented	Dented	Dented	Dented	Dented	Enteric
Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
Surface colour	Smooth	Smooth	Smooth	Smooth	Smooth	Rough	Smooth	Smooth
Pigmentation	Creamy	Creamy	Creamy	Creamy	Creamy	Pinkish	Pinkish	Creamy
G-stain	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Probable organism	1 clostridium sprogenes	2 Aerococcs Viridams	3 Streptococcs lactic epidemis	4 Micrococcs luteus	5 Staphylococucs luteus	6 Streptococcs lactic faecalis	7 Streptococcs lactic faecalis	

KEY

\+ = Positive

\- = Negative

A = Acid Production

The identified characterization was in line with the works of other scholars [37,42,44,45]. The result of this study clearly showed that the organisms had biodegradable abilities and values of degraded crude oil that varied after day 7 and 14. The total colony counts for day 1,4,8 and 14 are shown on Table 3. At day 1, the highest colony count was four (4). By day 4, the highest colony count was seven (7); at day 8, sixteen (16) was recorded, but by day 14 the highest colony count recorded was seventy (70). The result showed that the bacterial culture carryout a maximum degradation percentage of crude oil after 14 days of incubation. Most of the bacteria isolated have been proven to biodegrade a different range of petroleum hydrocarbon components [37,43,44]. During the screening of hydrocarbon degrading bacteria from the 10 core selected isolates; all the isolates (1, 2,3,4,5,6,7,8,14 and 18) were able to grow, utilizing crude as their carbon source. This corresponds to the findings of previous scholars [37,47]. Isolate 1, 4, 8 and 14 most especially, all produced clear zones ranging from 2 to 4 clear zones to multiple clear zones during the testing of the ability of the isolates to degrade crude oil. The findings of this study agree with the works of Nwakanma [37]; Okerentugba and Ezeronye [48]; and Mansi [44].

TABLE 2: Culture and Microscope Characterization of Fungi Isolate

Cultural characteristics	Microscopic	Identification
White mycelia growth on PDA after 24 hours.	Cordidiophore hyaline slender upper part sparingly branch conidia.	Articlospara inflate
Submerge aquatic with branched septate mycelium, simple cordidiophore slender hyaline.	Whitish cotton like mycelia which turns red on PDA plate.	Dendropspora erecta
Black mycelia on culture media after 48 hours.	Chain of conididial bonne on phial ides with black glucose head supported by septet was observed.	Aspergillus Niger
Whitish mycelia which later turns grey on APDA plate.	Mycelia external conidiophores upright simple upper portion which increases in length as conidia formed.	Oidioderium species
White septate mycelia on PDA plate	Conidia arthrospore hyaline J. celled shut cylindrical with truncate end.	Geotrichum albidum
Gray mycelia on PDA Plate which were dusty.	Conidiophores upright simple terminating in a globule or elevate swelling bearing phralites at apex.	Aspergillus fumigates
Dark mycelia on PDA plate.	Loosely tall mycelia tall conidiophores branch spirally coiled.	Streptothric Atra

TABLE 3: Total colony count in Agar media

Day 1

Microorganism	10^1	10^4	10^8
Clostridium Sprogenes	2	2	2
Aerococcus Viridams	2	3	4
Streptococcus lactic	-	1	2
Micrococcus luteus	1	3	2
Streptococcus epidemidis	-	2	4
Streptococcus faecalis	2	1	3
Bacillus anthracis	-	1	4

Day 4

Microorganism	10^1	10^4	10^8
Clostridium Sprogenes	3	2	3
Aerococcus Viridams	5	1	5
Streptococcus lactic	2	2	4
Micrococcus luteus	4	2	3

Streptococcus epidemidis	6	3	5
Streptococcus faecalis	5	3	7
Bacillus anthracis	7	2	6

Day 8

Microorganism	**10^1**	**10^4**	**10^8**
Clostridium Sprogenes	7	5	11
Aerococcus Viridams	9	10	16
Streptococcus lactic	4	8	14
Micrococcus luteus	6	4	11
Streptococcus epidemidis	8	9	4
Streptococcus faecalis	9	6	8
Bacillus anthracis	15	10	12

Day 14

Microorganism	**10^1**	**10^4**	**10^8**
Clostridium Sprogenes	9	8	20
Aerococcus Viridams	15	17	30
Streptococcus lactic	8	7	22
Micrococcus luteus	11	14	7
Streptococcus epidemidis	15	9	16
Streptococcus faecalis	10	13	22
Bacillus anthracis	70	11	17

5. Conclusion

The availability of petroleum hydrocarbons in any environment has been reported to influence the biodiversity, distribution and pollution of microorganisms [37]. Crude oil, despite its numerous advantages to the economy of any nation [13]; it is also one of the most significant pollutants in the environment that is capable of causing serious devastation to the ecosystem and human health [33,37,46]. Remediation of petroleum polluted sites in the subsurface environment is a real-world problem [14,31,33,37]. However, there are now biological control solutions to remove hazardous elements from the environment; as microbial remediation process has been reported as a successful and safe way to enhance environmental health in particular with low cost, technique and high public acceptance to cleaning up aquatic ecosystems from oil spills [37].

It has been reported by previous scholars that the environment of microorganisms in the degradation of petroleum has been established to be efficient, economical, versatile and environmentally friendly for treatment of petroleum polluted sites [37,44]. Thus, we conclude that bioremediation method can be effectively used to clean up the petroleum polluted sites in Bodo West as the available conditions can encourage the growth and multiplication of hydrocarbon utilizing bacteria.

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