Screening of Hydrocarbon Degrading Bacteria in Municipal Drainage in the Niger Delta

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ABSTRACT
Bioremediation has been severally described in literature as eco-friendly, cost effective and efficient remediation technology. The technology makes use of the capabilities of certain microorganisms such as bacteria and fungi to biodegrade certain organic pollutant such petroleum hydrocarbon. The availability of the right species and sufficient population of the hydrocarbon degrading bacteria is crucial to any successful bioremediation process. Biosludge is reported to contain important micronutrient and a wide array of microorganisms. In this study, certain petroleum hydrocarbon degrading bacteria has been isolated and identified from municipal drainage biosludge. The biosludge was collected from municipal drainage in Emeyal 2 community in Ogbia Local Government Area of Bayelsa State within the Niger Delta region of Nigeria. The Hydrocarbon degrading bacteria isolated and identified by biochemical methods include Pseudomonas sp, Serratia sp, Staphylococcus sp, Corrybacterium sp, Enterobacter spp, Micrococcusp, Flavobacterium, Achromobacter, Escherichia coli, Enterococcus sp and Citrobacter sp. A total of eleven different bacteria species were isolated and identified. This shows that the biosludge is a good medium for bioremediation as most of the organisms have been proven to biodegrade different range of petroleum hydrocarbon constituents.

Keyword: *bioremediation, bacteria, isolation, characterization, degradation and eco friendly*

1.1 INTRODUCTION.

Biosludge also known as excess sludge, biological sludge, secondary sludge or activated sludge is produced aerobically in biological treatment such as microbial digestion of organic matter in water (Sydney water, 20012). E.g, microbial digestion of organic matter in municipal drainage, sewage, domestic and waste water treatment processes. Biosludge mainly consists of micro-organisms and adsorbed suspended solids and colloids. Biological sludge is said to contain significant amount of organic matter which concentration varies

between 60 and 80%, with a typical value of 75% (Sydney water, 20012).. Biosludge is produce from biological processes such as in food processing industry, wine and bear production process, industrial and wastewater treatment processes, from agricultural residues such as sludge water from conventional animal feeding operations (CAFO) and community sewage as in municipal drainage.

The biosludge from industrial processes such as paper mill and wine production can be comparable to communal biosludge although it has more wood based ingredients like lignin compounds and absorbed chlorine compounds. (Lietteiden et al, 2001; Pham, 2011 and Jaakko, 2014). . Biosludge in municipal drainage is made up of domestic waste water, kitchen sewage, street run off organic and inorganic matters and microorganisms. Biosludge is been use in various ways for the ultimate benefit of man and society in general in some countries .For example, biosludge is use as farm amendment medium, in land filling as land reclamation medium, in road construction as road base material in Australia. It is also use in co-generation of power production and energy recovery in Australia and New Zealand. They also use it as source of biofuel, in production of bricks and construction material and in landfill capping etc. (www.biolids.comau/whatax.biosolid. In Nigeria, Municipal drainage biosludge has been a challenge that municipal residents and environmental sanitation authority have to deal with as it reduce free flow of water and can even lead to blockage of drainage. Up to this moment, no beneficial use of municipal drainage biosludge has been developed in Nigeria even though it contains some plant nutrient and certain microorganisms such as bacteria and fungi which can be environmentally beneficial. Bioremediation has been severally described in literature as eco-friendly, cost effective and efficient remediation technology. The technology takes advantage of the capabilities of certain microorganisms such as bacteria and fungi to biodegrade, bio transform and detoxify certain organic pollutant in the environment. The availability of certain species of bacteria are key to the biodegradation of hydrocarbon in the polluted environment. Apart from the availability of the bacteria the availability of favourable condition such as nutrient, sufficient population of the bacteria are also important factors for successful bioremediation process. This study is basically aimed at isolating and identifying potential hydrocarbon degrading bacteria in municipal drainage biosludge obtained from Emeyal 2 community in Ogbia Local Government Area of Bayelsa State in the Niger Delta.

2.1 MATERIALS AND METHOD

2.1.1 STUDY AREA.

Drainage biosludge for this study was collected from municipal in Emeyal 2 Community in Ogbia Local Government Area of Bayelsa State. Emeyal 2 shares boundaries with Imiringi, Elebele and Emeyal 1. It Was the colonial headquarter of Emeyal District during colonial administration in Nigeria. Till date, the community still serve as the headquarter of Emeyal Clan in the Ogbia Local Government Area of Bayelsa State. Politically, Emeyal 2 is the Headquarter of constituency 3 which is one of the three electoral constituencies in Ogbia. The community is host to oil (Karibol Opuberi families) well operated by Shell Petroleum Development Company (SPDC) l. Emeyal 2 is one the biggest and well planned communities in Bayelsa State. Ogbia in Emeyal 2 is situated, is located in the Southern part of Bayelsa State, lying within $4^{o}93'00^{//}$ $N16^{o}00^{//}E$ and occupying a land Area of about 695km2, with a population of about 179,926. The indigenous people of Emeyal 2 community are mostly Fishermen/ farmers and business men.

2.2.1 Materials and Method.

2.2.2 Materials

The materials used in for this research include: shovel, recyclable polythene bags, sack bags, ethanol, microscope and glass slide lactophenol blue stain and microbial culture media test tubes and test tube-rack, staining rack, sterilizing pot, petri dishes, oven aluminum foil and incubator etc.

2.2.3 Methodology.

The biosludge samples for this research were collected from three different sampling points in concrete drainage along the major road of Emeyal 2 community in Ogbia Local Government Area in Bayelsa State. The sludge was scoope from the drainage with shovel onto the road pavement and allowed for the water to drain before being put into clean and sterilized polythene bags and transported to the laboratory for biological analysis.

2.2.4 Preparation

Nutrient media were prepared by the following procedure.

Glass petri dishes were washed thoroughly and dried in an oven then sterilized wrapped in aluminium foil and kept at 160^{o}C for one hour.

- The test tubes and pipette were washed and dried like the petri dishes.
- The media ie (Bushnell Haas, Sabouraud Dextrose Agar and nutrient Agar) were prepared according to specification of the procedures in literature and for the Bushnell Haas; 20g of Agar Agar was added to every 1000ml. After sterilization, few drops of sterile crude oil were added to it which serves as sole source of carbon for hydrocarbon utilizing bacteria.
- The Bushnell Haas media prepared were incorporated with 10mglml keterazole to inhibit fungal growth and chloramphenicol to inhibit bacteria growth.

2.2.5 Bacteria Isolation and enumeration.

The populations of microorganisms in biosludge samples were isolated and enumerated using serial dilution pour plate method of Pepper and Gerba (2004), Benson (2002).

- About 1g of the biosludeg sample was weighed into 9ml sterile distilled water and shaked properly.
- The dilution was made up to 10^{-1} and serially diluted to $10^{-8.}$
- 1ml of each diluent was inoculated into the sterilized petri dish.
- The prepared /sterilized media was poured into the petri dishcontaining the inoculums.
- The petri dish was rotated on the bench several times (clockwise and anticlockwise) to achieve evenly spreading of the inoculum.
- The plate was allowed to stand/set was incubated turning them upside down at 37^{o}C for one week for the hydrocarbon utilizing microbes, 3-5 days for total fungi and 30oC for 24hours for total heterotrophic bacteria.
- The plate was examined after 5 days for hydrocarbon degrading bacteria a and after 24hours for total heterotrophic bacteria and the colony growth in each plate were counted using magnifying hand lens.
- The plates showing between 30-300 colonies were recorded.
- From the counting, the total viable microbial cells in the sample were expressed as colony forming unit per gram of each sample (CFU/g).
- Colonies different in size, shape and colour were selected from different agar plates and sub cultured for further analysis.

Calculation of total colony forming units was done using this formula.

$$\text{Colony forming unit/g of sample} = \frac{\text{No of colonies x reciprocal of the dilution factor}}{\text{Aliquor}}$$

2.2.6 Bacteria identification.

The biosludge samples were streaked on MacConkey Agar, Blood Agar and Mannitol salt Agar and the resultant colonies were further sub cultured in nutrient Agar Before biochemical tests were carried out. The bacteria isolates were identified by biochemical test (gram reaction, motility, indole, catalase, methyl red, Voges-Proskaur, coagulase, oxidase, urease and citrate). The resultant characteristics were compared with those of known taxas using Bergey's manual of Determinate Bacteriology by Holt et al, (1994) and the scheme of Cheesbrough (2004)

Table 1: result of biochemical charaterisation of bacteria in the biosludge sample

Organisms	**Gram reaction**	**Motility**	**Oxidase**	**Catalase**	**Citrate**	**Coagulate**	**Urease**	**Indole**	**Methyl red**	**VP**	**Hemolytic**	**H_2S**
Pseudomonas sp	Negative rod	+	+	+	+	--	--	--	--	+	NA	--
Serratia sp	Negative rod	+	+	+	+	--	--	--	NA	NA	NA	NA
Bacillus sp	Positive rod	+	+	+	+	--	--	--	--	+	NA	+
Staphylococcus sp	Positive rod	--	--	+	--	+	--	--	+	+	NA	+
Corrybacterium sp	Positive rod	--	--	+	+	--	--	+	NA	NA	NA	NA
Enterobacter spp	Negative rod	+	--	+	+	--	--	--	--	+	NA	--
Micrococcusp	Positive rod	--	--	+	--	--	--	--	+	--	NA	--
Flavobacterium	Negative rod	--	--	+	NA	--	NA	+	+	--	NA	+
Achromobacter	Negative	+	+	+	+	NA	+	--	NA	--	NA	NA

	rod											
Alcaligenes	Negative rod	+	+	+	+	NA	NA	--	NA	NA		NA
Escherichia	Negative rod		--	+	--	--	--	+	+	--	NA	NA
Enterococcus sp	Positive rod	--	--	+	NA	--	NA	--	--	--	NA	+
Citrobacter sp	Negative rod	+	--	+	+	--	NA	--	+	--	NA	NA

(+ = positive, - negative reaction, NA= Not applicable)
Courtesy: Laboratory analysis.

Table 2: Bacteria isolates from the samples of biosludge.

Location1	Location2	Location3
Pseudomonas sp	Pseudomonas sp	Pseudomonas sp
Serratia sp	--	--
Bacillus sp	Bacillus sp	Bacillus sp
Staphylococcus sp	--	Staphylococcus sp
Corrybacterium sp	Corrybacterium sp	Corrybacterium sp
Enterobacter spp	Enterobacter spp	Enterobacter spp
Micrococcusp	Micrococcusp	Micrococcusp
Flavobacterium	Flavobacterium	Flavobacterium
Achromobacter	Achromobacter	Achromobacter
--	Alcaligenes	Alcaligenes
Escherichia coli	Escherichia coli	Escherichia coli
Enterococcus sp	Enterococcus sp	Enterococcus sp
	Citrobacter sp	Citrobacter sp

Viable count of bacterial in the biosludge samples

Table 3: Microbial density of the various samples.

MICROBES	**Biosludge Samples location**		
	1	2	3
Total heterotrophic bacteria	$8.6x10^6$	$4.9x10^6$	$7.5x10^6$
Hydrocarbon utilizing bacteria	$3.9x10^4$	$8.2x10^4$	$9.1x10^4$

3.1 Discussion.

Bioremediation has been described by many scholars in literature as eco-friendly, cost effective and efficient remediation technology. The technology takes advantage of the capabilities of certain microorganisms such as bacteria fungi and plants to biodegrade, biotransform and detoxifies certain organic pollutant in the environment. The availability of certain species of bacteria are key to the biodegradation of hydrocarbon in the polluted environment. Apart from the availability of the bacteria the availability of favourable conditions such nutrient and sufficient population of the bacteria is also important factors for successful bioremediation process.

In this study, isolation and identification of hydrocarbon degrading bacteria in municipal drainage biosludge obtained from a community in Bayelsa State in the Niger Delta region of Nigeria has been carried out. The result shows that the municipal drainage biosludge obtained from the community (Emeyal 2) in Ogbia Local Government Area of Bayelsa State contain a rich consortium of hydrocarbon degrading bacteria. The Hydrocarbon degrading bacteria isolated and identified by biochemical methods include, Pseudomonas sp, Serratia sp, Staphylococcus sp, Corrybacterium sp, Enterobacter spp , Micrococcusp, Flavobacterium, Achromobacter, Escherichia coli, Enterococcus sp and Citrobacter sp. A total of eleven different bacteria species were solated and identified. This shows that the biosludge is a good medium for bioremediation as most of the organisms have been proven to biodegrade different range of petroleum hydrocarbon constituents. For example, Pseudomonas sp is shown to biodegrade Benzene, toluene, ethylbenzene,xylene, naphthalene, phenanthrene, kerosene and diesel (Watanabe, 2001; Joshi and Pandy, 2011), Bacillus degrade toluene and diesel (Joshi and Pandy, 2011), Alcaligenes degrade most PAHs (not specific) (Mao et al., 2012), Micrococcus biodegrade low molecular weight PAHs. (Othman et al., 2011), Cornebacterium also biodegrade low molecular weight PAHs. (Othman et al., 2011), Flavobacterium also have been discovered to biodegrade PAHs (not specific) (Li et al., 2009). Etc. The total population of heterotrophic bacterium enumerated per gram of each biosludge sample collected are $8.6x10^6$; $4.9x10^6$ and $7.5x10^6$ for station 1,2 and 3 respectively While total hydrocarbon degrading bacteria are $3.9x\ 10^4$, $8.2x10^4$ and $9.1x10^4$

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