

Research Article

Isolation and Characterization of Petroleum Hydrocarbon Degrading Fungi from Municipal Drainage Biosludge in The Niger Delta

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Abstract

Fungi and bacteria are known to effectively biodegrade petroleum hydrocarbon. Fungi are reported to be more efficient in Co-Metabolism of organic matters. In this study, isolation and characterization of petroleum hydrocarbon degrading fungi in municipal drainage bio-sludge has been undertaken by microscopic and macroscopic methods. Eight different strains of fungi were isolated and identified. These include *Aspergillus niger*, *Candida species*, *Penicillium species*, *Mucor species*, *Rhodotorulla species*, *Rhizopus* and *Trichoderma species*. and *Cladosporium species*. All these fungi have been shown by various studies to degrade divers type of Polycyclic Aromatic Hydrocarbon (PAHs). The population of total hetetrophic fungi identified ranges from 1.24×10^4 to 1.71×10^5 cfu/g while the population of hydrocarbon degradation fungi ranges from 2.1×10^3 to 9.71×10^4 cfu/g. The ability of Fungi to degrade different constituents of petroleum hydrocarbon make them an important element in biodegradation technology and this study has shown that municipal drainage biosludge obtained from the Niger Delta has the potential to be harnessed as a rich source of petroleum hydrocarbon degrading fungi. It is recommended that further research on petroleum hydrocarbon degrading fungi diversity in municipal drainage biosludge be undertaken with view to optimizing it use as bioremediation agent.

Keywords: Biodegrading; Bioremediation; Fungi; Isolation and characterization

Introduction

Biosludge is a slushy mass of settle able solid materials produce in waste water by the microbial degradation of organic material in the body of the waste water. It is mainly consisting of Micro-Organisms and adsorbed suspended solids and colloids which include both organic and inorganic materials [1]. The organic content of biological sludge is said to vary between 60 and 80%, with a typical average value of 75%. Biosludge are mainly produce from waste water treatment processes and some industrial and agricultural processes. Our municipal drainage is another major source of biosludge as organic and inorganic materials from streets and communities' neighborhoods are washed into the drainage by storm water during heavy rains. Also contributing to drainage biosludge are kitchen and domestic waste water from homes, restaurants and eateries.

Biosludge when treated or processed can be categories into three basic types depending on the nature of treatment. These include dewatered biosludge lime amended biosludge and composted

biosludge (Sydney Water) [1]. Information available in literature indicates that biosludge contains microorganisms and essential micro nutrients. This suggests that municipal drainage biosludge can be a potential habitat of hydrocarbon degrading microorganisms such as bacteria and fungi considering their ubiquitous nature in the environment.

Bioremediation is a pollution treatment technology that makes use of the ability of bacteria and fungi to biodegrade organic compound to cleanup hydrocarbon pollution. This technology offers the possibility of degrading, removing, altering, immobilizing, or otherwise detoxifying petroleum hydrocarbon from the environment through the action of bacteria fungi and plants [2-4]. There are different technologies that have been developed for the remediation of crude oil pollution. However, bioremediation technologies offer greater advantages because of its cost effectiveness and environmental friendliness [5-8]. Bacteria and fungi are among the major groups of microorganisms that are widely used in bioremediation applications of Chikere, et al. [9]. Fungi have proven useful in bioremediation of polluted environments and among their features which enable them to play critical role in bioremediation are, their ability to secret extracellular enzymes, ability to grow

under stressed environmental conditions, penetration into biomass location through hyphal growth, easy and rapid growth on agricultural or forest waste, and other enzyme systems Obire, et al. [10], George-Okafor, et al. [11].

Fungi are known to biodegrade organic matter by directly using the organic matter as a source of carbon and by enzymatically attacking the substrate but not using it as carbon source (Co-Metabolism). However, fungi are reported to be more efficient in co-metabolism of organic matters. These group of microbes are unique organisms due to their morphological, physiological and genetic characteristics. Fungi are a group of microorganisms scientifically regarded as non-green plants. They are environmentally versatile and known to inhabit all forms of environmental media (soil, water, sediment and air). They play important role in maintaining ecosystem balance. Different species of fungi have been shown to degrade different components of petroleum hydrocarbon ranging from straight chain hydrocarbon to complex polycyclic aromatic hydrocarbon. The focus of this study is the isolation and morphological/biochemical characterization of petroleum hydrocarbon degrading fungi from municipal drainage biosludge collected from a typical community in the Niger Delta region of Nigeria.

Materials and Method

Study Area

Drainage biosludge for this study was collected from municipal drainage in Emeyal 2 Community in Ogbia Local Government Area of Bayelsa State. Emeyal 2 Was the colonial headquarter of Emeyal District, it still serves 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 constituencies in Ogbia. The community is also a host to Shell Petroleum Development Company of Nigeria (SPDC) oil well bordered by Elebele and Imiringi communities. Emeyal 2 is one the biggest and well planned communities in Ogbia Local Government Area. Ogbia is located in the Southern part of Bayelsa State, lying within 4°93'00" N 16°00' E and occupying a land Area of about 695 km², with a population of about 179,926 according to the 1996 census. The inhabitants of Emeyal 2 community are mostly fishermen/ farmers and business men.

Tools and Apparatus

The materials used in for this research include shovel, trowel, recyclable polythene bags, cover all and sack bags for putting the collected biosludge. Scientific apparatus includes, petri dishes, glass slide, Pasteur pipette, Erlenmeyer's flask, test tubes, test tube rack, incubator, staining rack, pressure cooker, oven and microscope.

Chemicals and Culture Media

The chemicals and culture media used for the study include

95% Ethanol, lactophenol, Blue stain, Chloramphenicol, Bushnell Haas broth agar, Nutrient Agar, Sabouraud Dextrose Agar. The composition of the Bushnell Haas broth agar includes MgSO₄ (0.2 g/l), KH₂PO₄ (1 g/l), K₃HPO₄ (1 g/l), CaCl₂ (0.02 g/l), NH₄NO₂ (1 g/l) and FCl₂ (0.05 g/l). The composition of, nutrient agar, Peptide digest (5 g/l), Beef extract (5 g/l), Yeast extract (1.5 g/l), NaCl (5 g/l), and Agar (1.5 g/l) pH (7.4). The composition Sabouraud Dextrose Agar include Dextrose (40 g/l), Peptone (10 g/l) and Agar (15 g/l)

Method

The biosludge was collected from three different locations in concrete drainage along the major road of Emeyal 2 community in Ogbia Local Government Area in Bayelsa State. The collected sludge was allowed to dewater before putting into clean and sterilized polythene bags and transported to the laboratory for biological analysis.

Biological Analysis

Biological analysis was carried out on the biosludge samples to isolate and identify Fungi inherent in the samples. After isolation, total heterotrophic fungi and total Hydrocarbon Utilizing Fungi (HUF) were enumerated and identified by morphological and biochemical examination.

Isolation, Enumeration and Identification

Total heterotrophic and total hydrocarbon utilizing fungi in the biosludge samples were isolated, enumerated and identified by microbiological methods.

The fungi were isolated from the samples by culturing them under growth conditions of media (Sabouraud Dextrose Agar and nutrient Agar). 1 g of each biosludge samples was weighed and dissolve in sterilized distilled water and properly shaken. Each of the resultant mixture was made to 10⁻¹. Each of the diluents was further serially diluted up to 10⁻⁸. 1 ml of each diluent was then inoculated into sterilized petri dish. The prepared media was poured into each of the Petri dishes containing biosludge water inoculums. The plates were rotated in clockwise and anticlockwise direction to ensure uniform spread of the inoculums. The plates were allowed to set and then incubated in upside down position at 37 °C for one week. The plates were observed with magnifying hand lens for hydrocarbon degrading fungal growth after 3 to 5 days. The plates showing between 30 to 300 colonies were recorded.

Microscopic Identification of Fungi

Fragment of a well grown culture was immerse in 0.1ml of 95% ethanol on a glass slide. The ethanol was allowed to evaporate and the specimen stained with 0.1ml lactophenol blue stain. The stained specimen was then covered with cover slide and viewed microscopically. The resultant morphology was compared using the guide provided by Pepper [12-15].

Organisms	Microscopic morphology	Macroscopic morphology
<i>Aspergillus niger</i>	Presence of septate hyphae, black long, smooth, erect conidiophores Hyalineichoromously branched vesicle round, radiate head.	Brownish-black mycelium with dark pores on the surface
<i>Aspergillus fumigatus</i>	Presence of rough conidiophores, with uni/biseriate phialides whose vesicle is round with radiate head	Presence of blue-green to yellow coloration from surface.
<i>Aspergillus flavus</i>	Presence of septate hyphae, long smooth, colorless and sometimes brownish conidiophores with round radiate head vesicles and biseriate phialides.	A yellow-green color with a creamy edge appears on the surface, which appears golden to reddish brown on the reverse side.
<i>Candida species</i>	Single clusters of blastoconidia which is round and elongate. Long branched pseudohyphae were also observed.	A creamy to yellowish colonies with smooth, pasty, glistening or dry, wrinkled and dull color.
<i>Penicillium species</i>	Presence of red pigment with edges surrounded by whitish margin. Also the conidiophores are branched. Septate and fruity mycelium is observed.	A bluish-green filament is seen which changes to powdery greenish brown. It has brush phialospores arrangement
<i>Mucor species</i>	Presence of visible spore and short sporangiospores with non-septate hyphae.	A slimy colonies of texture with dark pigmented spores.
<i>Fusarium species</i>	Presence of dark pigment of micro and macro conidiophores.	Presence of sickle-shaped macroconidia that is yellow to purple in color.
<i>Rhodotorulla</i>	Spherical to elongate budding yeast-like cells or blastoconidia.	Colonies are coral red to salmon-coloured or slightly orange, smooth to wrinkled, highly glossy to semi-glossy.
<i>Rhizopus</i>	Presence of stolon's and pigmented rhizoids, the formation of sporangiospores, singly or in groups from nodes directly above the rhizoids and apophysate, columellate, multipored, generally globose sporangia	Presence of zygomycetes spores with dark pigment colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellow brown with sporulation.
<i>Trichoderma</i>	Presence of conidiophores repeatedly branched irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask-shaped phialides.	Presence of green conidia filament that resemble penicillin conidia are mostly green, sometimes. Hypae with smooth or rough walls and are formed in slimy conidial heads (gloriospora) clustered at the tips of the phialids
<i>Cladosporium</i>	Microscopy shows ascending to erect olivaceous-green apically branched, elongate conidiophores producing branched acropetal chains of smooth-walled conidia	Presence of light to grayish surface, gray to black surface blastoconidia

Table 1: Microscopic and macroscopic morphology of Fungi isolates.

Results

Isolated and identified fungi in biosludge samples

Samples locations		
Location 1	Location 2	Location 3
<i>Aspergillus Niger</i>	<i>Aspergillus Niger</i>	<i>Aspergillus Niger</i>
<i>Candida species</i>	-	<i>Candida species</i>
<i>Penicillium species</i>	<i>Penicillium species</i>	<i>Penicillium species</i>
<i>Mucor species</i>	<i>Mucor species</i>	<i>Mucor species</i>

<i>Rhodotorulla species</i>	<i>Rhodotorulla species</i>	<i>Rhodotorulla species</i>
<i>Rhizopus</i>	<i>Rhizopus</i>	<i>Rhizopus</i>
<i>Trichoderma species</i>	<i>Trichoderma species</i>	<i>Trichoderma species</i>
-	<i>Cladosporium species</i>	<i>Cladosporium species</i>

Table 2: Fungi isolates from the three biosludge sample.

Population of Fungi Enumeration

Biosludge Soil Samples			
MICROBES	Location1	Location 2	Location 3
Total heterotrophic Fungi	1.24x10 ⁴	2.09x10 ⁴	1.71x10 ⁵
Hydrocarbon utilizing Fungi	6.8x10 ⁴	2.1x10 ³	9.71x10 ⁴

Table 3: Fungi density in each of the biosludge samples.

Discussion

Biodegradation strategies are generally divided into two, these strategies include (1) The one in which the target compound is directly used as carbon source and (2) the strategy in which the target compound is enzymatically attacked but not used as carbon source i.e.(Co-Metabolism). Fungi are known to participate in both strategies of biodegradation, however fungi are reported to be more efficient in co-metabolism of organic matters. Fungi are unique organisms due to their morphological, physiological and genetic characteristics. They are environmentally versatile and known to inhabit all forms of environmental media (soil, water and air). Fungi help to play important role in maintaining ecosystem balance. Different species of fungi have been shown to degrade different components of petroleum hydrocarbon ranging from straight chain hydrocarbon to complex polycyclic aromatic hydrocarbon. In this study, isolation and characterizations of heterophilic and hydrocarbon degrading fungi in municipal drainage biosludge obtained from Emeyal 2 community in Ogbia Local Government Area of Bayelsa State has been carried out. Microscopic and macroscopic morphological features were used to identify the fungi. Seven different species of fungi were isolated and identified from samples collected from three different locations within the drainage. He reported that many of the fungal genera isolated and identified from this study have been implicated in degradation of hydrocarbons such as crude oil, polyromantic hydrocarbons and refined petroleum. Genera identified in this study include *Aspergillus Niger*; *Candida* spp, *Rhodotorulla*, *Rhizopus*, *Trichoderma* spp, *Cladosporium* spp and *Mucor* spp, isolated and characterized Eight strains of Hydrocarbon degrading fungi from crude oil polluted soil in the Niger Delta, which include all the fungi identified in this study. Filamentous fungi such as *Aspergillus* spp. and *Penicillium* spp have been reported as the predominance fungi in crude oil polluted environments by (April, et al. [5], D' Annibale,

et al. [16], Das, et al. [8] and Chikere, et al. [9]). *Penicillium* species, *Rhodotorulla*, *Rhizopus* and others have been shown to degrade various type of Polycyclic Aromatic Hydrocarbon (PAHs). The ability of Fungi to degrade different components of petroleum hydrocarbons make them an important element in biodegradation technology. The population of Total heterotrophic fungi identified from the three sampling stations ranges from 1.24x10⁴ to 1.71x10⁵ cfu/g. while hydrocarbon degradation fungi ranges from 2.1x10³ to 9.71x10⁴ cfu/g. Since Fungi has been discovered to play important role in the biodegradation of simple and complex petroleum hydrocarbons and many other organic pollutant, their bioavailability in municipal drainage can be harness to control the widespread crude oil pollution the Niger Delta.

Conclusion

Bioremediation has been proven to be an effective, cheaper and environmentally friendly and hydrocarbon pollution remediation technology. However, this technology can only work when certain microorganisms such as fungi and bacteria are present in the polluted media such as soil, water or sediment in appropriate population or they are introducing into the polluted media by bio augmentation. The results obtained in this study has shown that municipal drainage biosludge collected in the Niger Delta Region of Nigeria is a rich source of hydrocarbon degrading fungi which can be harnessed for pollution remediation.

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